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<p>(21) International Application Number: PCT/CA94/00604</p> <p>(22) International Filing Date: 1 November 1994 (01.11.94)</p> <p>(30) Priority Data:</p> <table> <tr><td>08/154,490</td><td>18 November 1993 (18.11.93)</td><td>US</td></tr> <tr><td>08/154,693</td><td>18 November 1993 (18.11.93)</td><td>US</td></tr> <tr><td>08/154,694</td><td>18 November 1993 (18.11.93)</td><td>US</td></tr> <tr><td>08/190,617</td><td>2 February 1994 (02.02.94)</td><td>US</td></tr> </table> <p>(71) Applicant (<i>for all designated States except US</i>): WESTAIM TECHNOLOGIES INC. [CA/CA]; Box 1000, 10101 - 114 Street, Fort Saskatchewan, Alberta T8L 2P2 (CA).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (<i>for US only</i>): BURRELL, Robert Edward [CA/CA]; 112 Village Downs, Sherwood Park, Alberta T8A 4L6 (CA). APTE, Prasad, Shrikrishna [CA/CA]; 34 Langholm Drive, St. Albert, Alberta T8N 3W1 (CA). GILL, Kashmir, Singh [CA/CA]; 58 Malvern Drive, Sherwood Park, Alberta T8A 3W1 (CA). PRECHT, Roderick, John [CA/CA]; 7877 - 26 Avenue, Edmonton, Alberta T6K 3S7 (CA). MORRIS, Larry, Roy [CA/CA]; Rural Route #2, Yarker, Ontario K0K 3N0 (CA). MCINTOSH, Catherine, Laurie [CA/CA]; 713 Georgian Terrace, Sherwood Park, Alberta T8A 2T7 (CA). SANT, Sudhindra, Bharat [CA/CA];</p>		08/154,490	18 November 1993 (18.11.93)	US	08/154,693	18 November 1993 (18.11.93)	US	08/154,694	18 November 1993 (18.11.93)	US	08/190,617	2 February 1994 (02.02.94)	US	<p>3431 - 139 Avenue #215, Edmonton, Alberta T5Y 1Z4 (CA).</p> <p>(74) Agent: OGILVIE AND COMPANY; #1400 Metropolitan Place, 10303 Jasper Avenue, Edmonton, Alberta T5J 3N6 (CA).</p> <p>(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).</p>	
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<p>Published <i>With international search report.</i></p>															
<p>(54) Title: ANTI-MICROBIAL MATERIALS</p> <p>(57) Abstract</p> <p>Anti-microbial coatings and powders and method of forming same on medical devices are provided. The coatings are preferably formed by depositing an anti-microbial biocompatible metal by vapour deposition techniques to produce atomic disorder in the coating such that a sustained release of metal ions sufficient to produce an anti-microbial effect is achieved. Preferred deposition conditions to achieve atomic disorder include a lower than normal substrate temperature, and one or more of a higher than normal working gas pressure and a lower than normal angle of incidence of coating flux. Anti-microbial powders formed by vapour deposition or altered by mechanical working to produce atomic disorder are also provided. The anti-microbial effect of the coatings or powders may be further activated or enhanced by irradiating with a low linear energy transfer form of radiation such as gamma radiation. Novel anti-microbial silver materials are defined, characterized by having a positive rest potential, a T_{ref}/T_m less than 0.33, and a grain size less than 200 nm. Anti-microbial fine grain or nanocrystalline materials are provided, together with methods of preparation, wherein the anti-microbial metal is deposited in a matrix with atoms or molecules of a different material such as other biocompatible metals (ex. Ta), trapped or absorbed oxygen, or compounds of anti-microbial metals or biocompatible metals (ex. Ag₀ or TaO). The invention also extends to a method of producing an anti-microbial effect with silver materials that form complex silver ions other than Ag⁺, Ag²⁺ and Ag³⁺.</p>															

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1

"ANTI-MICROBIAL MATERIALS"

2

FIELD OF THE INVENTION

3

The invention relates to methods of forming anti-microbial metal coatings, foils and powders which provide a sustained release of anti-microbial metal species when in contact with an alcohol or electrolyte.

6

BACKGROUND OF THE INVENTION

7

The need for an effective anti-microbial coating is well established in the medical community. Physicians and surgeons using medical devices and appliances ranging from orthopaedic pins, plates and implants through to wound dressings and urinary catheters must constantly guard against infection. An inexpensive anti-microbial coating also finds application in medical devices used in consumer healthcare and personal hygiene products as well as in biomedical/biotechnical laboratory equipment. The term "medical device", as used herein and in the claims is meant to extend to all such products.

14

The anti-microbial effects of metallic ions such as Ag, Au, Pt, Pd, Ir (i.e. the noble metals), Cu, Sn, Sb, Bi and Zn are known (see Morton, H.E., *Pseudomonas in Disinfection, Sterilization and Preservation*, ed. S.S. Block, Lea and Febiger, 1977 and Grier, N., *Silver and Its Compounds in Disinfection, Sterilization and Preservation*, ed. S.S. Block, Lea and Febiger, 1977). Of the metallic ions with anti-microbial properties, silver is perhaps the best known due to its unusually good bioactivity at low concentrations. This phenomena is termed oligodynamic action. In modern medical practice both inorganic and organic soluble salts of silver are used to prevent and treat microbial infections. While these compounds are effective as soluble salts, they do not provide prolonged protection due to loss through removal or complexation of the free silver ions. They must be

1 reapplied at frequent intervals to overcome this problem. Reapplication is not always
2 practical, especially where an in-dwelling or implanted medical device is involved.

3 Attempts have been made to slow the release of silver ions during treatment
4 by creating silver containing complexes which have a lower level of solubility. For
5 example, U.S. Patent 2,785,153 discloses colloidal silver protein for this purpose. Such
6 compounds are usually formulated as creams. These compounds have not found wide
7 applicability in the medical area due to their limited efficacy. The silver ion release rate
8 is very slow. Furthermore, coatings from such compounds have been limited due to
9 adhesion, abrasion resistance and shelf life problems.

10 The use of silver metal coatings for anti-microbial purposes has been
11 suggested. For instance, see Deitch et al., Anti-microbial Agents and Chemotherapy, Vol.
12 23(3), 1983, pp. 356 - 359 and Mackeen et al., Anti-microbial Agents and Chemotherapy,
13 Vol. 31(1), 1987, pp. 93 - 99. However, it is generally accepted that such coatings alone
14 do not provide the required level of efficacy, since diffusion of silver ions from the
15 metallic surface is negligible.

16 A silver metal coating is produced by Spire Corporation, U.S.A. under the
17 trade mark SPI-ARGENT. The coating is formed by an ion-beam assisted deposition
18 (IBAD) coating process. The infection resistant coating is stated to be non-leaching in
19 aqueous solutions as demonstrated by zone of inhibition tests, thus enforcing the belief that
20 silver metal surfaces do not release anti-microbial amounts of silver ions.

21 Given the failure of metallic silver coatings to generate the required anti-
22 microbial efficacy, other researchers have tried novel activation processes. One technique
23 is to use electrical activation of metallic silver implants (see Marino et al., Journal of
24 Biological Physics, Vol. 12, 1984, pp. 93 - 98). Electrical stimulation of metallic silver

1 is not always practical, especially for mobile patients. Attempts to overcome this problem
2 include developing in situ electrical currents through galvanic action. Metal bands or
3 layers of different metals are deposited on a device as thin film coatings. A galvanic cell
4 is created when two metals in contact with each other are placed in an electrically
5 conducting fluid. One metal layer acts as an anode, which dissolves into the electrolyte.
6 The second metal acts as a cathode to drive the electrochemical cell. For example, in the
7 case of alternating layers of Cu and Ag, the Cu is the anode, releasing Cu⁺ ions into the
8 electrolyte. The more noble of the metals, Ag, acts as the cathode, which does not ionize
9 and does not go into solution to any large extent. An exemplary device of this nature is
10 described in U.S. Patent 4,886,505 issued Dec. 12, 1989, to Haynes et al. The patent
11 discloses sputtered coatings of two or more different metals with a switch affixed to one
12 of the metals such that, when the switch is closed, metal ion release is achieved.

13 Previous work has shown that a film composed of thin laminates of
14 alternating, different metals such as silver and copper can be made to dissolve if the
15 surface is first etched. In this instance, the etching process creates a highly textured
16 surface (see M. Tanemura and F. Okuyama, J. Vac. Sci. Technol., 5, 1986, pp 2369-2372).
17 However, the process of making such multilaminated films is time consuming and
18 expensive.

19 Electrical activation of metallic coatings has not presented a suitable solution
20 to the problem. It should be noted that galvanic action will occur only when an electrolyte
21 is present and if an electrical connection between the two metals of the galvanic couple
22 exists. Since galvanic corrosion occurs primarily at the metallic interface between the two
23 metals, electrical contact is not sustained. Thus a continuous release of metal ions over
24 an extended period of time is not probable. Also, galvanic action to release a metal such

1 as silver is difficult to achieve. As indicated above, the metal ions exhibiting the greatest
2 anti-microbial effect are the noble metals, such as Ag, Au, Pt and Pd. There are few
3 metals more noble than these to serve as cathode materials so as to drive the release of a
4 noble metal such as Ag at the anode.

5 A second approach to activating the silver metal surface is to use heat or
6 chemicals. U.S. Patents 4,476,590 and 4,615,705, issued to Scales et al. on October 16,
7 1984 and October 7, 1986, respectively, disclose methods of activating silver surface
8 coatings on endoprosthetic implants to render them bioerodible by heating at greater than
9 180°C or by contacting with hydrogen peroxide. Such treatments are limited in terms of
10 the substrate/devices which can be coated and activated.

11 There is still a need for an efficacious, inexpensive anti-microbial material
12 having the following properties:

- 13 - sustained release of an anti-microbial agent at therapeutically active levels;
14 - applicable to a wide variety of devices and materials;
15 - useful shelf life; and
16 - low mammalian toxicity.

17 Metal coatings are typically produced as thin films by vapour deposition
18 techniques such as sputtering. Thin films of metals, alloys, semiconductors and ceramics
19 are widely used in the production of electronic components. These and other end uses
20 require the thin films to be produced as dense, crystalline structures with minimal defects.
21 The films are often annealed after deposition to enhance grain growth and recrystallization
22 and produce stable properties. Techniques to deposit metal films are reviewed by R.F.
23 Bunshah et al., "Deposition Technologies for Films and Coatings", Noyes Publications,
24 N.J., 1982 and by J.A. Thornton, "Influence of Apparatus Geometry and Deposition

1 Conditions on the Structure and Topography of Thick Sputtered Coatings", J. Vac. Sci.
2 Technol., 11(4), 666-670, 1974.

3 U.S. Patent No. 4,325,776, issued April 20, 1982 to Menzel discloses a
4 process for producing coarse or single crystal metal films from certain metals for use in
5 integrated circuits. The metal film is formed by depositing on a cooled substrate (below
6 90°C) such that the metal layer is in an amorphous phase. The metal layer is then
7 annealed by heating the substrate up to about room temperature. The end product is stated
8 to have large grain diameter and great homogeneity, permitting higher current densities
9 without electromigration failures.

10 Silver salts such as those of nitrate, proteins, acetate, lactate and citrate have
11 been suggested for use in anti-microbial coatings for medical devices. Silver nitrate is
12 used in burn wound dressings in many hospitals. These salts are known to have better
13 anti-microbial efficacy than silver metal. The mechanism by which these compounds are
14 effective is the instant ionization/dissociation to produce the Ag⁺ ion. The availability of
15 the Ag⁺ ion is reduced significantly within or in contact with bodily fluids or tissues. Due
16 to the high chloride content of such fluids, the silver is precipitated or tied up as insoluble
17 silver chloride ($K_{sp} = 1.7 \times 10^{-10} M$). As a consequence, excessive amounts of silver must
18 be present within any media containing precipitants (chiefly chloride) in order to produce
19 the same efficacy from a silver salt as would be observed in water.

20 Nanocrystalline materials in the forms of powders, films and flakes are
21 materials which are single-phase or multi-phase polycrystals, the grain size of which is in
22 the order of a few (typically <20) nanometers in at least one dimension. Fine grain
23 powders (particle size <5 microns) may be nanocrystalline, or more typically have grain
24 sizes >20 nm. Nanocrystalline materials and fine powders may be prepared by a number

1 of modified gas condensation methods, wherein the material to be deposited is generated
2 in the vapour phase, for example by evaporation or sputtering, and is transported into a
3 relatively large volume in which the working gas atmosphere and temperature is controlled.
4 Atoms of the material to be deposited collide with atoms of the working gas atmosphere,
5 lose energy and are rapidly condensed from the vapour phase onto a cold substrate, such
6 as a liquid nitrogen cooled finger. In principle, any method capable of producing very fine
7 grain sized polycrystalline materials can be used to produce nanocrystalline materials.
8 These methods include, for example, evaporation such as arc evaporation, electron beam
9 vapor deposition, molecular beam epitaxy, ion beam, sputtering, magnetron sputtering and
10 reactive sputtering (see for example, Froes, F.H. et al., "Nanocrystalline Metals for
11 Structural Applications", JOM, 41 (1989), No. 6., pp 12 - 17; Birninger, Rainer et al.,
12 "Nanocrystalline Materials - A First Report, Proceedings of JIMIS-4; and Gleiter, H.
13 "Materials with Ultrafine Microstructures: Retrospectives and Perspectives,
14 NanoStructured Materials, Vol. 1, pp 1-19, 1992, and references cited therein).

15 **SUMMARY OF THE INVENTION**

16 The inventors set out to develop an anti-microbial metal coating. They
17 discovered that, contrary to previous belief, it is possible to form metal coatings from an
18 anti-microbial metal material by creating atomic disorder in the materials by vapour
19 deposition under conditions which limit diffusion, that is which "freeze-in" the atomic
20 disorder. The anti-microbial coatings so produced were found to provide sustained release
21 of anti-microbial metal species into solution so as to produce an anti-microbial effect.

22 This basic discovery linking "atomic disorder" to enhanced solubility has
23 broad application. The inventors have demonstrated that atomic disorder so as to produce

1 solubility can be created in other material forms, such as metal powders. The invention
2 also has application beyond anti-microbial metals, encompassing any metal, metal alloy,
3 or metal compound, including semiconductor or ceramic materials, from which sustained
4 release of metal species into solution is desired. For instance, materials having enhanced
5 or controlled metal dissolution find application in sensors, switches, fuses, electrodes, and
6 batteries.

7 The term "atomic disorder" as used herein includes high concentrations of:
8 point defects in a crystal lattice, vacancies, line defects such as dislocations, interstitial
9 atoms, amorphous regions, grain and sub grain boundaries and the like relative to its
10 normal ordered crystalline state. Atomic disorder leads to irregularities in surface
11 topography and inhomogenieties in the structure on a nanometre scale.

12 By the term "normal ordered crystalline state" as used herein is meant the
13 crystallinity normally found in bulk metal materials, alloys or compounds formed as cast,
14 wrought or plated metal products. Such materials contain only low concentrations of such
15 atomic defects as vacancies, grain boundaries and dislocations.

16 The term "diffusion" as used herein implies diffusion of atoms and/or
17 molecules on the surface or in the matrix of the material being formed.

18 The terms "metal" or "metals" as used herein are meant to include one or
19 more metals whether in the form of substantially pure metals, alloys or compounds such
20 as oxides, nitrides, borides, sulphides, halides or hydrides.

21 The invention, in a broad aspect extends to a method of forming a modified
22 material containing one or more metals. The method comprises creating atomic disorder
23 in the material under conditions which limit diffusion such that sufficient atomic disorder
24 is retained in the material to provide release, preferably on a sustainable basis, of atoms,

1 ions, molecules or clusters of at least one of the metals into a solvent for the material.
2 Clusters are known to be small groups of atoms, ions or the like, as described by R.P.
3 Andres et al., "Research Opportunities on Clusters and Cluster-Assembled Materials", J.
4 Mater. Res. Vol. 4, No. 3, 1989, P. 704.

5 Specific preferred embodiments of the invention demonstrate that atomic
6 disorder may be created in metal powders or foils by cold working, and in metal coatings
7 by depositing by vapour deposition at low substrate temperatures.

8 In another broad aspect, the invention provides a modified material
9 comprising one or more metals in a form characterized by sufficient atomic disorder such
10 that the material, in contact with a solvent for the material, releases atoms, ions, molecules
11 or clusters containing at least one metal, preferably on a sustainable basis, at an enhanced
12 rate relative to its normal ordered crystalline state.

13 In preferred embodiments of the invention, the modified material is a metal
14 powder which has been mechanically worked or compressed, under cold working
15 conditions, to create and retain atomic disorder.

16 The term "metal powder" as used herein is meant to include metal particles
17 of a broad particle size, ranging from nanocrystalline powders to flakes.

18 The term "cold working" as used herein indicates that the material has been
19 mechanically worked such as by milling, grinding, hammering, mortar and pestle or
20 compressing, at temperatures lower than the recrystallization temperature of the material.
21 This ensures that atomic disorder imparted through working is retained in the material.

22 In another preferred embodiment, the modified material is a metal coating
23 formed on a substrate by vapour deposition techniques such as vacuum evaporation,
24 sputtering, magnetron sputtering or ion plating. The material is formed under conditions

1 which limit diffusion during deposition and which limit annealing or recrystallization
2 following deposition. The deposition conditions preferably used to produce atomic
3 disorder in the coatings are outside the normal range of operating conditions used to
4 produce defect free, dense, smooth films. Such normal practices are well known (see for
5 example R.F. Bunshah et al., supra). Preferably the deposition is conducted at low
6 substrate temperatures such that the ratio of the substrate to the melting point of the metal
7 or metal compound being deposited (T/T_m) is maintained at less than about 0.5, more
8 preferably at less than about 0.35, and most preferably at less than 0.30. In this ratio, the
9 temperatures are in degrees Kelvin. The preferred ratio will vary from metal to metal and
10 increases with alloy or impurity content. Other preferred deposition conditions to create
11 atomic disorder include one or more of a higher than normal working gas pressure, a lower
12 than normal angle of incidence of the coating flux and a higher than normal coating flux.

13 The temperature of deposition or cold working is not so low that substantial
14 annealing or recrystallization will take place when the material is brought to room
15 temperature or its intended temperature for use (ex. body temperature for anti-microbial
16 materials). If the temperature differential between deposition and temperature of use (ΔT)
17 is too great, annealing results, removing atomic disorder. This ΔT will vary from metal
18 to metal and with the deposition technique used. For example, with respect to silver,
19 substrate temperatures of -20 to 200°C are preferred during physical vapour deposition.

20 Normal or ambient working gas pressure for depositing the usually required
21 dense, smooth, defect free metal films vary according to the method of physical vapour
22 deposition being used. In general, for sputtering, the normal working gas pressure is less
23 than 10 Pa (Pascal) (75 mT (milliTorr)), for magnetron sputtering, less than 1.3 Pa (10mT),
24 and for ion-plating less than 30 Pa (200 mT). Normal ambient gas pressures vary for

1 vacuum evaporation processes vary as follows: for e-beam or arc evaporation, from
2 0.0001 Pa (0.001 mT) to 0.001 Pa (0.01 mT); for gas scattering evaporation (pressure
3 plating) and reactive arc evaporation, up to 30 Pa (200 mT), but typically less than 3 Pa
4 (20 mT). Thus, in accordance with the method of the present invention, in addition to
5 using low substrate temperatures to achieve atomic disorder, working (or ambient) gas
6 pressures higher than these normal values may be used to increase the level of atomic
7 disorder in the coating.

8 Another condition discovered to have an effect on the level of atomic
9 disorder in the coatings of the present invention is the angle of incidence of the coating
10 flux during deposition. Normally to achieve dense, smooth coatings, this angle is
11 maintained at about $90^{\circ} \pm 15^{\circ}$. In accordance with the present invention, in addition to
12 using low substrate temperatures during deposition to achieve atomic disorder, angles of
13 incidence lower than about 75° may be used to increase the level of atomic disorder in the
14 coating.

15 Yet another process parameter having an effect on the level of atomic
16 disorder is the atom flux to the surface being coated. High deposition rates tend to
17 increase atomic disorder, however, high deposition rates also tend to increase the coating
18 temperature. Thus, there is an optimum deposition rate that depends on the deposition
19 technique, the coating material and other process parameters.

20 To provide an anti-microbial material, the metals used in the coating or
21 powder are those which have an anti-microbial effect, but which are biocompatible (non-
22 toxic for the intended utility). Preferred metals include Ag, Au, Pt, Pd, Ir (i.e. the noble
23 metals), Sn, Cu, Sb, Bi, and Zn, compounds of these metals or alloys containing one more
24 of these metals. Such metals are hereinafter referred to as "anti-microbial metals"). Most

1 preferred is Ag or its alloys and compounds. Anti-microbial materials in accordance with
2 this invention preferably are formed with sufficient atomic disorder that atoms, ions,
3 molecules or clusters of the anti-microbial material are released into an alcohol or water
4 based electrolyte on a sustainable basis. The terms "sustainable basis" is used herein to
5 differentiate, on the one hand from the release obtained from bulk metals, which release
6 metal ions and the like at a rate and concentration which is too low to achieve an anti-
7 microbial effect, and on the other hand from the release obtained from highly soluble salts
8 such as silver nitrate, which release silver ions virtually instantly in contact with an alcohol
9 or water based electrolyte. In contrast, the anti-microbial materials of the present invention
10 release atoms, ions, molecules or clusters of the anti-microbial metal at a sufficient rate
11 and concentration, over a sufficient time period to provide a useful anti-microbial effect.

12 The term "anti-microbial effect" as used herein means that atoms, ions,
13 molecules or clusters of the anti-microbial metal are released into the electrolyte which the
14 material contacts in concentrations sufficient to inhibit bacterial growth in the vicinity of
15 the material. The most common method of measuring anti-microbial effect is by
16 measuring the zone of inhibition (ZOI) created when the material is placed on a bacterial
17 lawn. A relatively small or no ZOI (ex. less than 1 mm) indicates a non-useful anti-
18 microbial effect, while a larger ZOI (ex. greater than 5 mm) indicates a highly useful anti-
19 microbial effect. One procedure for a ZOI test is set out in the Examples which follow.

20 The invention extends to devices such as medical devices formed from,
21 incorporating, carrying or coated with the anti-microbial powders or coatings. The anti-
22 microbial coating may be directly deposited by vapour deposition onto such medical
23 devices as catheters, sutures, implants, burn dressings and the like. An adhesion layer,
24 such as tantalum, may be applied between the device and the anti-microbial coating.

1 Adhesion may also be enhanced by methods known in the art, for example etching the
2 substrate or forming a mixed interface between the substrate and the coating by
3 simultaneous sputtering and etching. Anti-microbial powders may be incorporated into
4 creams, polymers, ceramics, paints, or other matrices, by techniques well known in the art.

5 In a further broad aspect of the invention, modified materials are prepared
6 as composite metal coatings containing atomic disorder. In this case, the coating of the
7 one or more metals or compounds to be released into solution constitutes a matrix
8 containing atoms or molecules of a different material. The presence of different atoms or
9 molecules results in atomic disorder in the metal matrix, for instance due to different sized
10 atoms. The different atoms or molecules may be one or more second metals, metal alloys
11 or metal compounds which are co- or sequentially deposited with the first metal or metals
12 to be released. Alternatively the different atoms or molecules may be absorbed or trapped
13 from the working gas atmosphere during reactive vapour deposition. The degree of atomic
14 disorder, and thus solubility, achieved by the inclusion of the different atoms or molecules
15 varies, depending on the materials. In order to retain and enhance the atomic disorder in
16 the composite material, one or more of the above-described vapour deposition conditions,
17 namely low substrate temperature, high working gas pressure, low angle of incidence and
18 high coating flux, may be used in combination with the inclusion of different atoms or
19 molecules.

20 Preferred composite materials for anti-microbial purposes are formed by
21 including atoms or molecules containing oxygen, nitrogen, hydrogen, boron, sulphur or
22 halogens in the working gas atmosphere while depositing the anti-microbial metal. These
23 atoms or molecules are incorporated in the coating either by being absorbed or trapped in
24 the film, or by reacting with the metal being deposited. Both of these mechanisms during

1 deposition are hereinafter referred to as "reactive deposition". Gases containing these
2 elements, for example oxygen, hydrogen, and water vapour, may be provided continuously
3 or may be pulsed for sequential deposition.

4 Anti-microbial composite materials are also preferably prepared by co- or
5 sequentially depositing an anti-microbial metal with one or more inert biocompatible
6 metals selected from Ta, Ti, Nb, Zn, V, Hf, Mo, Si, and Al. Alternatively, the composite
7 materials may be formed by co-, sequentially or reactively depositing one or more of the
8 anti-microbial metals as the oxides, carbides, nitrides, borides, sulphides or halides of these
9 metals and/or the oxides, carbides, nitrides, borides, sulphides or halides of the inert
10 metals. Particularly preferred composites contain oxides of silver and/or gold, alone or
11 together with one or more oxides of Ta, Ti, Zn and Nb.

12 The invention also extends to a method of activating or further enhancing
13 the anti-microbial effect of anti-microbial materials formed with atomic disorder. Thus,
14 anti-microbial materials made in accordance with the present invention may be irradiated
15 to further enhance the anti-microbial effect. However, it is also possible to irradiate
16 materials initially formed with a level of atomic disorder which is insufficient to produce
17 an anti-microbial effect, such that the irradiated material has an acceptable anti-microbial
18 effect. The process of activation comprises irradiating the material with a low linear
19 energy transfer form of radiation such as beta or x-rays, but most preferably gamma rays.
20 A dose greater than 1 Mrad is preferred. The anti-microbial material is preferably oriented
21 substantially perpendicular to the incoming radiation. The level of activation may be
22 further enhanced by irradiating the material adjacent to a dielectric material such as oxides
23 of Ta, Al and Ti, but most preferably silicon oxide.

1 The invention also extends to the preparation of anti-microbial silver
2 materials which form complex silver ions other than Ag⁺, Ag²⁺ and Ag³⁺, in contact with
3 an alcohol or a water based electrolyte. The complex silver ions are found to have a
4 surprisingly greater anti-microbial efficacy than does the Ag⁺ ion released from the silver
5 salts of the prior art. Exemplary complex silver ions include Ag(CN)₂⁻, AgCN_(aq)(ion pair),
6 Ag(NH₃)₂⁺, AgCl₂⁻, Ag(OH)₂⁻, Ag₂(OH)₃⁻, Ag₃(OH)₄⁻ and Ag(S₂O₃)₂³⁻. Silver coatings,
7 powders, flakes and foils prepared with atomic disorder in accordance with the present
8 invention are exemplary of silver materials which release complex silver ions having anti-
9 microbial efficacy. Alternatively the silver materials may be prepared as solutions,
10 ointments, paints or suspensions containing the complex silver ions. Such silver materials
11 have wide application, for example as coatings for medical devices, in topical anti-
12 microbial compositions, in anti-fouling paints or coatings and as coatings for anti-microbial
13 filters.

14 Thus, in accordance with a broad aspect of the invention, there is provided
15 a method of producing an anti-microbial effect in an alcohol or a water based electrolyte
16 comprising, preparing a silver material such that it forms complex silver ions other than
17 Ag⁺, Ag²⁺ and Ag³⁺ in an amount so as to produce an anti-microbial effect in contact with
18 an alcohol or water-based electrolyte that is greater than that produced by an equivalent
19 amount of silver as Ag⁺; and bringing the silver material in contact with the surface,
20 alcohol or electrolyte to be treated so as to cause the release of the complex silver ions.

21 The invention further extends to fine grain anti-microbial materials in a fine
22 powder, film or flake form, comprising one or more anti-microbial metals or alloys or
23 compounds thereof, having a grain size less than 200 nm, in a fine powder, flake or film
24 form, characterized by sufficient atomic disorder such that the material, in contact with an

1 alcohol or a water based electrolyte, provides a sustained release of the atoms, ions,
2 molecules or clusters of at least one anti-microbial metal into the alcohol or water based
3 electrolyte at a concentration sufficient to provide a localized anti-microbial effect.

4 The anti-microbial material may be prepared by introducing the atomic
5 disorder into a pre-formed fine grain or nanocrystalline (<20 nm) powder, flakes or films
6 of one or more of the anti-microbial metals by mechanical working, for example by
7 compressing the material, under cold working conditions. Alternatively, the atomic
8 disorder may be created during the synthesis of fine grain or nanocrystalline materials
9 (films, flakes or powders) by vapour deposition techniques in which the anti-microbial
10 metal is co-, sequentially or reactively deposited in a matrix with atoms or molecules of
11 a different material under conditions such that atomic disorder is created and retained in
12 the matrix. The different material (or dopant) is selected from inert biocompatible metals,
13 oxygen, nitrogen, hydrogen, boron, sulphur, and halogens, and oxides, nitrides, carbides,
14 borides, sulphides and halides of either of both of an anti-microbial metal or a
15 biocompatible metal. Preferred biocompatible metals include Ta, Ti, Nb, B, Hf, Zn, Mo,
16 Si and Al. These different materials may be included with the anti-microbial metal in the
17 same or separate target, for example a target of Ag and/or silver oxides, which may further
18 contain, for example, Ta or tantalum oxides. Alternatively, the different material may be
19 introduced from the working gas atmosphere during vapour deposition, for example by
20 sputtering or reactive sputtering in an atmosphere containing atoms or molecules of the
21 different material such as oxygen.

22 The anti-microbial form of silver material prepared in accordance with the
23 process of the present invention has been physically characterized and has been found to
24 have the following novel characteristics:

1 - a positive rest potential, E_{rest} when measured against a saturated calomel
2 reference electrode (SCE), in 1 M potassium hydroxide;
3 - preferably a ratio of temperature of recrystallization to its melting point,
4 in degrees K, (T_{rec}/T_m), of less than about 0.33, and most preferably less than about 0.30;
5 - preferably a temperature of recrystallization less than about 140 °C;
6 - preferably, a grain size less than about 200nm, preferably less than 140
7 nm and most preferably less than 90 nm.

8 Each of these physical characteristics, with perhaps the exception of grain
9 size, is believed to be the result of the presence of atomic disorder in the material. The
10 characteristics are of assistance in identifying and distinguishing the silver materials of the
11 present invention from prior art materials or materials in their normal ordered crystalline
12 state. The preferred novel anti-microbial silver materials have been characterized , for
13 example by XRD, XPS and SIMS analysis, as comprising substantially pure silver metal,
14 when deposited in an inert atmosphere such as argon. However, when the working gas
15 atmosphere contains oxygen, the materials comprise a matrix of substantially pure silver
16 metal and one or both of, silver oxide and atoms or molecules of trapped or absorbed
17 oxygen. A further distinguishing feature of the materials of the present invention is the
18 presence of growth twins in the grain structure, visible from TEM analysis.

19 **BRIEF DESCRIPTION OF THE DRAWINGS**

20 Figure 1 is a TEM micrograph of a sputter deposited silver coating in
21 accordance with the invention, illustrating grain size and growth twin defects.

22 Figure 2 is a TEM micrograph of the film of Figure 1 after annealing,
23 showing larger grain size and the presence of annealing twins.

1

DESCRIPTION OF THE PREFERRED EMBODIMENTS

2

As above stated, the present invention has application beyond anti-microbial materials. However, the invention is disclosed herein with anti-microbial metals, which are illustrative of utility for other metals, metal alloys and metal compounds. Preferred metals include Al and Si, and the metal elements from the following groups of the periodic table: IIIB, IVA, VB, VIB, VIIIB, IIB, IIIA, IVA, and VA (excluding As) in the periods 4, 5 and 6, (see Periodic Table as published in Merck Index 10th Ed., 1983, Merck and Co. Inc., Rahway, N.J., Martha Windholz). Different metals will have varying degrees of solubility. However, the creation and retention of atomic disorder in accordance with this invention results in enhanced solubility (release) of the metal as ions, atoms, molecules or clusters into an appropriate solvent i.e. a solvent for the particular material, typically a polar solvent, over the solubility of the material in its normal ordered crystalline state.

14

The medical devices formed from, incorporating, carrying or coated with the anti-microbial material of this invention generally come into contact with an alcohol or water based electrolyte including a body fluid (for example blood, urine or saliva) or body tissue (for example skin, muscle or bone) for any period of time such that microorganism growth on the device surface is possible. The term "alcohol or water based electrolyte" also includes alcohol or water based gels. In most cases the devices are medical devices such as catheters, implants, tracheal tubes, orthopaedic pins, insulin pumps, wound closures, drains, dressings, shunts, connectors, prosthetic devices, pacemaker leads, needles, surgical instruments, dental prostheses, ventilator tubes and the like. However, it should be understood that the invention is not limited to such devices and may extend to other devices useful in consumer healthcare, such as sterile packaging, clothing and footwear,

1 personal hygiene products such as diapers and sanitary pads, in biomedical or biotechnical
2 laboratory equipment, such as tables, enclosures and wall coverings, and the like. The
3 term "medical device" as used herein and in the claims is intended to extend broadly to
4 all such devices.

5 The device may be made of any suitable material, for example metals,
6 including steel, aluminum and its alloys, latex, nylon, silicone, polyester, glass, ceramic,
7 paper, cloth and other plastics and rubbers. For use as an in-dwelling medical device, the
8 device will be made of a bioinert material. The device may take on any shape dictated by
9 its utility, ranging from flat sheets to discs, rods and hollow tubes. The device may be
10 rigid or flexible, a factor again dictated by its intended use.

11 Anti-Microbial Coatings

12 The anti-microbial coating in accordance with this invention is deposited as
13 a thin metallic film on one or more surfaces of a medical device by vapour deposition
14 techniques. Physical vapour techniques, which are well known in the art, all deposit the
15 metal from the vapour, generally atom by atom, onto a substrate surface. The techniques
16 include vacuum or arc evaporation, sputtering, magnetron sputtering and ion plating. The
17 deposition is conducted in a manner to create atomic disorder in the coating as defined
18 hereinabove. Various conditions responsible for producing atomic disorder are useful.
19 These conditions are generally avoided in thin film deposition techniques where the object
20 is to create a defect free, smooth and dense film (see for example J.A. Thornton, supra).
21 While such conditions have been investigated in the art, they have not heretofore been
22 linked to enhanced solubility of the coatings so-produced.

1 The preferred conditions which are used to create atomic disorder during the
2 deposition process include:

3 - a low substrate temperature, that is maintaining the surface to be coated
4 at a temperature such that the ratio of the substrate temperature to the melting point of the
5 metal (in degrees Kelvin) is less than about 0.5, more preferably less than about 0.35 and
6 most preferably less than about 0.3; and optionally one or both of:

7 - a higher than normal working (or ambient) gas pressure, i.e. for vacuum
8 evaporation: e-beam or arc evaporation, greater than 0.001 Pa (0.01 mT), gas scattering
9 evaporation (pressure plating) or reactive arc evaporation, greater than 3 Pa (20 mT); for
10 sputtering: greater than 10 Pa (75 mT); for magnetron sputtering: greater than about 1.3
11 Pa (10 mT); and for ion plating: greater than about 30 Pa (200 mT); and

12 - maintaining the angle of incidence of the coating flux on the surface to be
13 coated at less than about 75°, and preferably less than about 30°

14 The metals used in the coating are those known to have an anti-microbial
15 effect. For most medical devices, the metal must also be biocompatible. Preferred metals
16 include the noble metals Ag, Au, Pt, Pd, and Ir as well as Sn, Cu, Sb, Bi, and Zn or alloys
17 or compounds of these metals or other metals. Most preferred is Ag or Au, or alloys or
18 compounds of one or more of these metals.

19 The coating is formed as a thin film on at least a part of the surface of the
20 medical device. The film has a thickness no greater than that needed to provide release
21 of metal ions on a sustainable basis over a suitable period of time. In that respect, the
22 thickness will vary with the particular metal in the coating (which varies the solubility and
23 abrasion resistance), and with the degree of atomic disorder in (and thus the solubility of)
24 the coating. The thickness will be thin enough that the coating does not interfere with the

1 dimensional tolerances or flexibility of the device for its intended utility. Typically,
2 thicknesses of less than 1 micron have been found to provide sufficient sustained anti-
3 microbial activity. Increased thicknesses may be used depending on the degree of metal
4 ion release needed over a period of time. Thicknesses greater than 10 microns are more
5 expensive to produce and normally should not be needed.

6 The anti-microbial effect of the coating is achieved when the device is
7 brought into contact with an alcohol or a water based electrolyte such as, a body fluid or
8 body tissue, thus releasing metal ions, atoms, molecules or clusters. The concentration of
9 the metal which is needed to produce an anti-microbial effect will vary from metal to
10 metal. Generally, anti-microbial effect is achieved in body fluids such as plasma, serum
11 or urine at concentrations less than about 0.5 - 1.5 $\mu\text{g}/\text{ml}$.

12 The ability to achieve release of metal atoms, ions, molecules or clusters on
13 a sustainable basis from a coating is dictated by a number of factors, including coating
14 characteristics such as composition, structure, solubility and thickness, and the nature of
15 the environment in which the device is used. As the level of atomic disorder is increased,
16 the amount of metal ions released per unit time increases. For instance, a silver metal film
17 deposited by magnetron sputtering at $T/T_m < 0.5$ and a working gas pressure of about 0.9
18 Pa (7 mTorr) releases approximately 1/3 of the silver ions that a film deposited under
19 similar conditions, but at 4 Pa (30 mTorr), will release over 10 days. Films that are
20 created with an intermediate structure (ex. lower pressure, lower angle of incidence etc.)
21 have Ag release values intermediate to these values as determined by bioassays. This then
22 provides a method for producing controlled release metallic coatings in accordance with
23 this invention. Slow release coatings are prepared such that the degree of disorder is low
24 while fast release coatings are prepared such that the degree of disorder is high.

1 For continuous, uniform coatings, the time required for total dissolution will
2 be a function of film thickness and the nature of the environment to which they are
3 exposed. The relationship in respect of thickness is approximately linear, i.e. a two fold
4 increase in film thickness will result in about a two fold increase in longevity.

5 It is also possible to control the metal release from a coating by forming a
6 thin film coating with a modulated structure. For instance, a coating deposited by
7 magnetron sputtering such that the working gas pressure was low (ex. 2 Pa (15 mTorr))
8 for 50% of the deposition time and high (ex. 4 Pa (30 mTorr)) for the remaining time, has
9 a rapid initial release of metal ions, followed by a longer period of slow release. This type
10 of coating is extremely effective on devices such as urinary catheters for which an initial
11 rapid release is required to achieve immediate anti-microbial concentrations followed by
12 a lower release rate to sustain the concentration of metal ions over a period of weeks.

13 The substrate temperature used during vapour deposition should not be so
14 low that annealing or recrystallization of the coating takes place as the coating warms to
15 ambient temperatures or the temperatures at which it is to be used (ex. body temperature).
16 This allowable ΔT , that the temperature differential between the substrate temperature
17 during deposition and the ultimate temperature of use, will vary from metal to metal. For
18 the most preferred metals of Ag and Au, preferred substrate temperatures of -20 to 200°C
19 , more preferably -10°C to 100°C are used.

20 Atomic order may also be achieved, in accordance with the present
21 invention, by preparing composite metal materials, that is materials which contain one or
22 more anti-microbial metals in a metal matrix which includes atoms or molecules different
23 from the anti-microbial metals.

1 Our technique for preparing composite material is to co- or sequentially
2 deposit the anti-microbial metal(s) with one or more other inert, biocompatible metals
3 selected from Ta, Ti, Nb, Zn, V, Hf, Mo, Si, Al and alloys of these metals or other metal
4 elements, typically other transition metals. Such inert metals have a different atomic radii
5 from that of the anti-microbial metals, which results in atomic disorder during deposition.
6 Alloys of this kind can also serve to reduce atomic diffusion and thus stabilize the
7 disordered structure. Thin film deposition equipment with multiple targets for the
8 placement of each of the anti-microbial and inert metals is preferably utilized. When
9 layers are sequentially deposited the layer(s) of the inert metal(s) should be discontinuous,
10 for example as islands within the anti-microbial metal matrix. The final ratio of the anti-
11 microbial metal(s) to inert metal(s) should be greater than about 0.2. The most preferable
12 inert metals are Ti, Ta, Zn and Nb. It is also possible to form the anti-microbial coating
13 from oxides, carbides, nitrides, sulphides, borides, halides or hydrides of one or more of
14 the anti-microbial metals and/or one or more of the inert metals to achieve the desired
15 atomic disorder.

16 Another composite material within the scope of the present invention is
17 formed by reactively co- or sequentially depositing, by physical vapour techniques, a
18 reacted material into the thin film of the anti-microbial metal(s). The reacted material is
19 an oxide, nitride, carbide, boride, sulphide, hydride or halide of the anti-microbial and/or
20 inert metal, formed in situ by injecting the appropriate reactants, or gases containing same,
21 (ex. air, oxygen, water, nitrogen, hydrogen, boron, sulphur, halogens) into the deposition
22 chamber. Atoms or molecules of these gases may also become absorbed or trapped in the
23 metal film to create atomic disorder. The reactant may be continuously supplied during
24 deposition for codeposition or it may be pulsed to provide for sequential deposition. The

1 final ratio of anti-microbial metal(s) to reaction product should be greater than about 0.2.

2 Air, oxygen, nitrogen and hydrogen are particularly preferred reactants.

3 The above deposition techniques to prepare composite coatings may be used
4 with or without the conditions of lower substrate temperatures, high working gas pressures
5 and low angles of incidence previously discussed. One or more of these conditions is
6 preferred to retain and enhance the amount of atomic disorder created in the coating.

7 It may be advantageous, prior to depositing an anti-microbial in accordance
8 with the present invention, to provide an adhesion layer on the device to be coated, as is
9 known in the art. For instance, for a latex device, a layer of Ti, Ta or Nb may be first
10 deposited to enhance adhesion of the subsequently deposited anti-microbial coating.

11 Anti-Microbial Powders

12 Anti-microbial powders, including nanocrystalline powders and powders
13 made from rapidly solidified flakes or foils, can be formed with atomic disorder so as to
14 enhance solubility. The powders either as pure metals, metal alloys or compounds such
15 as metal oxides or metal salts, can be mechanically worked or compressed to impart
16 atomic disorder. This mechanically imparted disorder is conducted under conditions of
17 low temperature (i.e. temperatures less than the temperature of recrystallization of the
18 material) to ensure that annealing or recrystallization does not take place. The temperature
19 varies between metals and increases with alloy or impurity content.

20 Anti-microbial powders produced in accordance with this invention may be
21 used in a variety of forms, for instance in topical creams, paints or adherent coatings.
22 Alternatively, the powder may be incorporated into a polymeric, ceramic or metallic matrix
23 to be used as a material for medical devices or coatings therefor.

1 Fine Grain or Nanocrystalline Materials of Anti-Microbial Metals

2 Methods of forming fine grain or nanocrystalline materials from the vapour
3 phase are well known and documented in the literature. For instance, nanocrystalline
4 materials may be formed by a modified standard inert-gas condensation technique. The
5 material to be deposited is evaporated from an electrically heated boat or crucible into an
6 inert gas atmosphere such as argon or helium with a pressure of about 5 to 7 Torr. The
7 temperature of the boat has to be high enough to obtain a substantial vapour pressure of
8 the material of interest. For metals, a temperature about 100°C above the melting point
9 of the metal will typically provide an adequate vapour pressure. Due to interatomic
10 collisions with the working gas atmosphere atoms, the evaporated atoms of the material
11 lose their kinetic energy and condense onto a cold finger or substrate held at about 77 K
12 (liquid nitrogen cooled) in the form of a loose powder or friable flakes or film, the grain
13 size of which is less than about 20 nm. With respect to powders or flakes, a high vacuum
14 (less than 5×10^{-6} Pa) is restored and the powder or flakes are stripped off from the cold
15 finger and collected in a cold trap.

16 Fine grain materials are produced analogously in gas condensation/vapour
17 deposition processes, as is known in the art. This is typically achieved by altering the cold
18 finger or substrate temperature and the gas pressure to allow the particle to coarsen to the
19 desired size which is preferably under 5000 nm.

20 Fine powders/nanocrystalline powders of anti-microbial metals prepared in
21 accordance with the known prior art processes have been tested and found not to have
22 sufficient anti-microbial efficacy. In order to introduce atomic disorder into the materials
23 at a level which is sufficient to produce an anti-microbial effect, the anti-microbial metal,
24 alloy or compound to be deposited is co-, sequentially or reactively deposited in a matrix

1 with atoms or molecules of a different material (dopant) under conditions such that atomic
2 disorder is created and retained in the matrix. The different material is selected from inert
3 biocompatible metals, such as Ta, Ti, Nb, B, Hf, Zn, Mo, Si and Al, most preferably Ta,
4 Ti and Nb. Alternatively the different material is an oxide, nitride, carbide, boride,
5 sulphide or halide of either or both of an anti-microbial metal or of the biocompatible
6 metal. A further alternative is to introduce the different material from the working gas
7 atmosphere, either by reactive deposition or by absorbing or trapping atoms or molecules
8 from the working gas into the matrix. Working gas atmospheres containing oxygen,
9 nitrogen, hydrogen boron, sulphur and halogens may be used. Working gas atmospheres
10 including oxygen are most preferred, such that the matrix of anti-microbial metal includes
11 either or both of trapped oxygen and oxides of the anti-microbial metal.

12 A further technique for forming anti-microbial powders of the present
13 invention is to form coatings containing atomic disorder in the manner set out above onto
14 an inert, preferably biocompatible, particulate material such as talc, bentonite, cornstarch
15 or ceramics such as alumina. The particles may be coated by physical vapour deposition
16 techniques under conditions to create atomic disorder, as set forth above in respect of the
17 anti-microbial metal coatings. Alternatively, the powders can be coated by adapting a
18 vapour deposition process, for instance by passing a vapour of the anti-microbial material
19 through a fixed porous bed of the powders, by fluidizing the powder bed in the anti-
20 microbial metal vapour phase, or by letting the powder fall through a vapour of the anti-
21 microbial material. In all cases, the powder could be cooled and/or the working gas
22 atmosphere could be altered to include a different material (ex. oxygen), in order to
23 produce the desired degree of atomic disorder.

1 Activation of Anti-Microbial Materials

2 Irradiation of anti-microbial materials (powders, nanocrystalline powders,
3 foils, coatings or composite coatings of anti-microbial metals) which contain atomic
4 disorder formed by any of the above-described procedures, will further activate or enhance
5 the anti-microbial effect. Thus, even materials having a low level of atomic disorder may
6 be activated to an anti-microbial level.

7 Irradiation is performed with any low linear energy transfer form of
8 radiation, including beta, gamma and x-rays. Gamma radiation at a dose of 1 Mrad or
9 greater is preferred. Since gamma radiation is an acceptable method of sterilization of
10 medical devices, activation and sterilization may be achieved simultaneously through the
11 irradiation process of the present invention.

12 The irradiation step is preferably conducted such that the anti-microbial
13 material being irradiated is oriented generally perpendicular to the incoming radiation
14 (rather than parallel). A further enhancement of the anti-microbial effect can be achieved
15 by conducting the irradiation step with a dielectric material adjacent to, or preferably
16 sandwiched around the anti-microbial material. Exemplary dielectrics include oxides of
17 Si, Ti, Ta and Al. Silicon oxide surfaces are preferred. It is believed that the dielectric
18 material provides forward scattering of electrons into the anti-microbial coating.

19 Without being bound by the same it is believed that the irradiation step is
20 causing one or more of the following changes in the anti-microbial material:

- 21 1) creating further atomic disorder, such as point defects;
22 2) enhancing oxygen adsorption/chemisorption to the surface of the anti-microbial
23 material;
24 3) activating trapped dopant atoms or molecules such as oxygen to O⁺ or O₂; and

1 4) creating broken or dangling bonds at the surface.
2 With respect to the second and third proposed mechanisms, it is possible that oxygen
3 adsorption/chemisorption and/or activation creates a super saturated concentration of O₂,
4 O⁺ or O₂⁻ species in or on the anti-microbial metal surface, which results in the more rapid
5 dissolution of the anti-microbial metal or species thereof into an aqueous environment
6 through the generation of various chemical species of the anti-microbial metal, including
7 oxides and hydroxides.

8 Silver Materials Forming Complex Silver Ions

9 In accordance with the invention, silver materials are prepared which form
10 complex silver ions other than Ag⁺, Ag²⁺ and Ag³⁺, when the material is contacted with an
11 alcohol or a water based electrolyte. Exemplary complex silver ions shown to demonstrate
12 an anti-microbial effect include Ag(CN)₂⁻, AgCN_(aq)^(ion pair), Ag(NH₃)₂⁺, AgCl₂⁻, Ag(OH)₂⁻,
13 Ag₂(OH)₃⁻, Ag₃(OH)₄⁻ and Ag(S₂O₃)₂³⁻. These silver materials forming complex silver ions
14 have wide application, for instance, as anti-microbial coatings for medical devices, as anti-
15 microbial powders for medical or pharmaceutical use, as anti-fouling paints, coatings or
16 compositions, anti-microbial coatings for filters and the like.

17 It should be understood that the phrase "silver materials which form
18 complex silver ions other than Ag⁺, Ag²⁺ and Ag³⁺" as used herein and in the claims is not
19 intended to exclude silver materials which form one or more of Ag⁺, Ag²⁺ and Ag³⁺, ions
20 in addition to the complex silver ions when the material contacts an alcohol or a water
21 based electrolyte. The notation Ag⁺, Ag²⁺ and Ag³⁺- refers to these ions in solution and
22 includes solvated forms. The term complex silver ions as used herein and in the claims

1 is not intended to include silver ions stabilized with strong oxidizing agents, such as
2 persulphate and periodate, to prevent the reduction of the silver ions.

3 The anti-microbial coatings, powders and foils of the present invention,
4 when created with atomic disorder as above described, are exemplary of silver materials
5 which form complex silver ions other than Ag^+ so as to cause an anti-microbial effect. It
6 is believed that the complex silver ions which may be formed when such silver materials
7 contact an alcohol or water based electrolyte, are one or more of the negative ions
8 Ag(OH)_2^- , $\text{Ag}_2(\text{OH})_3^-$ and $\text{Ag}_3(\text{OH})_4^-$.

9 Silver materials which form complex silver ions may also be prepared by
10 bringing a silver metal, compound or salt into an environment containing excessive
11 amounts of a cationic, anionic or neutral species with which it is desired to complex silver.
12 For example, the negative complex silver ion AgCl_2^- can be generated by placing a silver
13 salt such as AgNO_3 in an aqueous medium with an elevated concentration of the Cl^- ion.
14 $\text{AgNO}_3/\text{NaCl}$ or AgCl/NaCl mixtures, solutions or suspensions can form the AgCl_2^- ion.
15 This AgCl_2^- ion may also be generated with mixtures of silver powder with NaCl .
16 Preferably the silver powder is one which is prepared in accordance with the present
17 invention so as to contain atomic disorder, but bulk silver may also be activated in this
18 manner. Bulk silver powder, fine grain (<140 nm) and nanocrystalline (<20 nm) powders
19 may be used. Similarly, the ion $\text{Ag}(\text{NH}_3)_2^+$ can be formed in aqueous solution by adding
20 silver salts to excess ammonium hydroxide. The ion $\text{Ag}(\text{S}_2\text{O}_3)_2^{3-}$ may be formed in
21 aqueous solution by adding silver salts to excess sodium thiosulphate. The ion $\text{Ag}(\text{CN})_2^-$
22 may be formed in aqueous solution by adding excess potassium cyanide to silver cyanide.

23 The silver materials forming complex silver ions may be formulated for use
24 in many forms, including for example, powders, suspensions, solutions, ointments or

1 coatings. For instance, a pharmaceutical composition to generate the AgCl₂⁻ ion can be
2 formulated as a mixture of the salts AgNO₃/NaCl or as a mixture of NaCl with a silver
3 powder, preferably one containing atomic disorder. These mixtures of the silver material
4 might be pre-formulated as a solution, suspension or ointment with a sterile aqueous or
5 saline solution and pharmaceutically acceptable carriers, diluents, excipients and the like.
6 Alternatively the silver material might be provided as the mixtures of silver powder/NaCl
7 salt or AgNO₃/NaCl, for later formulation by the end user.

8 Physical/Chemical Characteristics of Anti-Microbial Silver Material

9 The modified metal materials formed in accordance with the present
10 invention so as to contain atomic disorder which leads to enhanced release of the metal
11 species have novel physical characteristics when compared with materials in their normal
12 ordered crystalline state. Silver materials made in accordance with the present invention
13 have been characterized as having the following novel characteristics:

- 14 - a positive rest potential, E_{rest}, for example, when measured against a SCE
15 reference electrode in a 1 M KOH solution;
- 16 - preferably a ratio of temperature of recrystallization to melting temperature
17 less than 0.33, and most preferably less than 0.30;
- 18 - preferably a temperature of recrystallization less than about 140 °C; and
- 19 - preferably a grain size less than about 200nm, more preferably less than
20 140nm and most preferably less than 90nm.

21 Analysis of the silver materials by XRD, XPS and SIMS techniques
22 confirms the chemical nature and content of the film as silver metal, and in the event that
23 the material is formed with oxygen in the working gas atmosphere, one or both of silver

1 oxide and trapped oxygen. TEM analysis reveals growth twins in the silver material,
2 which are converted to annealed twins when the materials are annealed above the
3 temperature of recrystallization.

4 The invention is further illustrated by the following non-limiting examples.

5 Example 1

6 A medical suture material size 2/0, polyester braid was coated by magnetron
7 sputtering 20.3 cm diameter (8 in.) from planar silver and copper magnetron cathodes to
8 form an Ag-Cu-alloy on the surface to a thickness of 0.45 microns, using either argon gas
9 working pressures of 0.9 Pa (7 mTorr) or 4 Pa (30 mT) at 0.5 KW power and a T/Tm ratio
10 of less than 0.5. The total mass flow of gas was 700 sccm (standard cubic centimeters per
11 minute).

12 The anti-microbial effect of the coatings was tested by a zone of inhibition
13 test. Basal medium Eagle (BME) with Earle's salts and L-glutamine was modified with
14 calf/serum (10%) and 1.5 % agar prior to being dispensed (15 ml) into Petri dishes. The
15 agar containing Petri plates were allowed to surface dry prior to being inoculated with a
16 lawn of *Staphylococcus aureus* ATCC# 25923. The inoculant was prepared from Bactrol
17 Discs (Difco, M.) which were reconstituted as per the manufacturer's directions.
18 Immediately after inoculation, the materials or coatings to be tested were placed on the
19 surface of the agar. The dishes were incubated for 24 h at 37°C. After this incubation
20 period, the zone of inhibition was measured and a corrected zone of inhibition was
21 calculated (corrected zone of inhibition = zone of inhibition - diameter of the test material
22 in contact with the agar).

1 The results showed no zone of inhibition on the uncoated suture, a zone of
2 less than 0.5 mm around the suture coated at 0.9 Pa (7 mTorr) and a zone of 13 mm
3 around the suture coated at 4 Pa (30 mTorr). Clearly the suture coated in accordance with
4 the present invention exhibits a much more pronounced and effective anti-microbial effect.

5 Example 2

6 This example is included to illustrate the surface structures which are
7 obtained when silver metal is deposited on silicon wafers using a magnetron sputtering
8 facility and different working gas pressures and angles of incidence (i.e. the angle between
9 the path of the sputtered atoms and the substrate). All other conditions were as follows:
10 target was a 20.3 cm diameter planar silver magnetron cathode; power was 0.1 kW;
11 deposition rate was 200 Å/min; ratio of temperature of substrate (wafer) to melting point
12 of silver (1234°K), T/Tm was less than 0.3. Argon gas pressures of 0.9 Pa (7 mTorr) (a
13 normal working pressure for metal coatings) and 4 Pa (30 mTorr) were used with a total
14 mass flow of 700 sccm. Angles of incidence at each of these pressures were 90° (normal
15 incidence), 50° and 10°. The coatings had a thickness of about 0.5 microns.

16 The resulting surfaces were viewed by scanning electron microscope. As
17 argon gas pressure increased from 0.9 Pa (7 mTorr) to 4 Pa (30 mTorr) the grain size
18 decreased and void volume increased significantly. When the angle of incidence was
19 decreased, the grain size decreased and the grain boundaries became more distinct. At 0.9
20 Pa (7 mTorr) argon pressure and an angle of incidence of 10°, there were indications of
21 some voids between the grains. The angle of incidence had a greater effect on the surface
22 topography when the gas pressure was increased to 4 Pa (30 mTorr). At 90°, the grain size
23 varied from 60 - 150 nm and many of the grains were separated by intergrain void spaces

1 which were 15 - 30 nm wide. When the angle of incidence was decreased to 50°, the grain
2 size decreased to 30 - 90 nm and the void volume increased substantially. At 10°, the
3 grain size was reduced to about 10 - 60 nm and void volumes were increased again.

4 The observed nanometre scale changes in surface morphology and
5 topography are indications of atomic disorder in the silver metal. While not being bound
6 by the same, it is believed that such atomic disorder results in an increase in the chemical
7 activity due to increased internal stresses and surface roughness created by mismatched
8 atoms. It is believed that the increased chemical activity is responsible for the increased
9 level of solubility of the coatings when in contact with an electrolyte such as body fluid.

10 The anti-microbial effect of the coatings was evaluated using the zone of
11 inhibition test as set out in Example 1. Each coated silicon wafer was placed on an
12 individual plate. The results were compared to the zones of inhibition achieved when solid
13 silver (i.e. greater than 99% silver) sheets, wires or membranes were tested. The results
14 are summarized in Table 1. It is evident that the pure silver devices and the silver
15 sputtered coating at 0.9 Pa (7 mTorr) do not produce any biological effect. However, the
16 coatings deposited at a higher than normal working gas pressure, 4 Pa (30 mTorr),
17 demonstrated an anti-microbial effect, as denoted by the substantial zones of inhibition
18 around the discs. Decreasing the angle of incidence had the greatest effect on anti-
19 microbial activity when combined with the higher gas pressures.

1

Table I

2
3Anti-microbial effects of various silver and silver coated samples as determined using *Staphylococcus aureus*

4

Sample	Percent Silver	Angle of Deposition	Working Gas Pressure (mTorr)	Corrected Zone of Inhibition (mm)
Silver Sheet-rolled	99+	-	-	<0.5
Silver wire (.0045")	99+	-	-	<0.5
Silver membrane-cast	99+	-	-	<0.5
Sputtered thin film	99+	normal (90°)	0.9 (7)	<0.5
Sputtered thin film	99+	50°	0.9 (7)	<0.5
Sputtered thin film	99+	10°	0.9 (7)	<0.5
Sputtered thin film	99+	normal (90°)	4 (30)	6.3
Sputtered thin film	99+	50°	4 (30)	10
Sputtered thin film	99+	10	4 (30)	10

36

Example 3

37

Silicon wafers were coated by magnetron sputtering using 20.3 cm diameter planar silver and copper magnetron cathodes to produce an alloy of Ag and Cu (80:20) at normal incidence at working gas pressures of 0.9 Pa (7 mTorr) and 4 Pa (30 mTorr), all other conditions being identical to those set out in Example 2. As in Example 2, when the coatings were viewed by SEM, the coatings formed at high working gas pressure had

1 smaller grain sizes and larger void volumes than did the coatings formed at the lower
 2 working gas pressures.

3 Coatings which were similarly formed as a 50:50 Ag/Cu alloy were tested
 4 for anti-microbial activity with the zone of inhibition test set out in Example 1. The
 5 results are summarized in Table 2. Coatings deposited at low working gas pressure (0.9
 6 Pa (7 mTorr)) showed minimal zones of inhibition, while the coatings deposited at high
 7 working gas pressure (4 Pa (30 mTorr)) produced larger zones of inhibition, indicative of
 8 anti-microbial activity.

9
 10 Table 2
 11 The anti-microbial effect of various sputter deposited silver-copper alloys as determined using *Staphylococcus*
aureus

12 Sample	13 Percent	14 Angle of	15 Working Gas	16 Corrected
17	18 Silver	19 Deposition	20 Pressure	21 Zone of
22	23	24	25	26
1	50	normal (90°)	1.0 (7.5)	<0.5
2	50	normal (90°)	4 (30)	16
3	50	10	4 (30)	19

24 Example 4

25 A coating in accordance with the present invention was tested to determine
 26 the concentration of silver ions released into solution over time. One cm² silicon wafer
 27 discs were coated with silver as set forth in Example 2 at 0.9 Pa (7 mTorr) and 4 Pa (30
 28 mTorr) and normal incidence to a thickness of 5000 Å. Using the method of Nickel et

1 al., Eur. J. Clin. Microbiol., 4(2), 213-218, 1985, a sterile synthetic urine was prepared and
2 dispensed into test tubes (3.5 ml). The coated discs were placed into each test tubes and
3 incubated for various times at 37°C. After various periods of time, the discs were removed
4 and the Ag content of the filtered synthetic urine was determined using neutron activation
5 analysis.

6 The results are set forth in Table 3. The table shows the comparative
7 amounts of Ag released over time from coatings deposited on discs at 0.9 Pa (7 mTorr)
8 or 4 Pa (30 mTorr). The coatings deposited at high pressure were more soluble than those
9 deposited at low pressure. It should be noted that this test is a static test. Thus, silver
10 levels build up over time, which would not be the case in body fluid where there is
11 constant turn over.

12 Table 3
13 Concentration of silver in synthetic urine as a function of exposure time

Silver Concentration $\mu\text{g}/\text{ml}$		
Exposure Time (Days)	Working Argon gas pressure 0.9 Pa (7mTorr)	Working argon gas pressure 4 Pa (30mTorr)
0	ND1	ND
1	0.89	1.94
3	1.89	2.36
10	8.14	23.06

28 Note: Films were deposited at normal incidence (90°)
29 1 - ND (non detectable) <0.46 $\mu\text{g}/\text{ml}$

1 Example 5

This example is included to illustrate coatings in accordance with the present invention formed from another noble metal, Pd. The coatings were formed on silicon wafers as set forth in Example 2, to a thickness of 5000 Å⁰, using 0.9 Pa (7 mTorr) or 4 Pa (30 mTorr) working gas pressures and angles of incidence of 90° and 10°. The coated discs were evaluated for anti-microbial activity by the zone of inhibition test substantially as set forth in Example 1. The coated discs were placed coating side up such that the agar formed a 1 mm surface coating over the discs. The medium was allowed to solidify and surface dry, after which the bacterial lawn was spread over the surface. The dishes were incubated at 37°C for 24 h. The amount of growth was then visually analyzed.

The results are set forth in Table 4. At high working gas pressures, the biological activity of the coating was much greater than that of coatings deposited at low pressure. Changing the angle of incidence (decreasing) improved the anti-microbial effect of the coating to a greater extent when the gas pressure was low than when it was high.

Table 4

16 Surface Control of Staphylococcus aureus by Sputter Deposited Palladium metal

	Sample	Sputtering Pressure Pa (mTorr)	Angle of Deposition	Anti-microbial Control
1	0.9 (7)	90°(normal incidence)	More than 90% of surface covered by bacterial growth	
2	0.9 (7)	10°(grazing incidence)	20-40% of surface covered by bacterial growth	
3	4 (30)	90°(normal incidence)	Less than 10% surface covered by bacterial growth	

1 Example 6

2 This example is included to illustrate the effect of silver deposition
3 temperature on the anti-microbial activity of the coating. Silver metal was deposited on
4 2.5 cm sections of a latex Foley catheter using a magnetron sputtering facility. Operating
5 conditions were as follows; the deposition rate was 200 A⁰ per minute; the power was 0.1
6 kW; the target was a 20.3 cm diameter planar silver magnetron cathode; the argon working
7 gas pressure was 4 Pa (30mTorr); the total mass flow was 700 sccm; and the ratio of
8 temperature of substrate to melting point of the coating metal silver, T/Tm was 0.30 or
9 0.38. In this example the angles of incidence were variable since the substrate was round
10 and rough. That is the angles of incidence varied around the circumference and, on a finer
11 scale, across the sides and tops of the numerous surface features. The anti-microbial effect
12 was tested by a zone of inhibition test as outlined in Example 1.

13 The results showed corrected zones of inhibition of 0.5 and 16 mm around
14 the tubing coated at T/Tm values of 0.38 and 0.30 respectively. The sections of Foley
15 catheter coated at the lower T/Tm value were more efficacious than those coated at higher
16 T/Tm value.

17 Example 7

18 This example is included to demonstrate an anti-microbial coating formed
19 by DC magnetron sputtering on a commercial catheter. A teflon coated latex Foley
20 catheter was coated by DC magnetron sputtering 99.99% pure silver on the surface using
21 the conditions listed in Table 5. The working gases used were commercial Ar and 99/1
22 wt% Ar/O₂.

1 The anti-microbial effect of the coating was tested by a zone of inhibition
2 test. Mueller Hinton agar was dispensed into Petri dishes. The agar plates were allowed
3 to surface dry prior to being inoculated with a lawn of *Staphylococcus aureus* ATCC#
4 25923. The inoculant was prepared from Bactrol Discs (Difco, M.) which were
5 reconstituted as per the manufacturer's directions. Immediately after inoculation, the
6 coated materials to be tested were placed on the surface of the agar. The dishes were
7 incubated for 24 hr. at 37°C. After this incubation period, the zone of inhibition was
8 measured and a corrected zone of inhibition was calculated (corrected zone of inhibition
9 = zone of inhibition - diameter of the test material in contact with the agar).

10 The results showed no zone of inhibition for the uncoated samples and a
11 corrected zone of less than 1 mm for catheters sputtered in commercial argon at a working
12 gas pressure of 0.7 Pa (5 mT). A corrected zone of inhibition of 11 mm was reported for
13 the catheters sputtered in the 99/1 wt% Ar/O₂ using a working gas pressure of 5.3 Pa (40
14 mT). XRD analysis showed that the coating sputtered in 1% oxygen was a crystalline Ag
15 film. This structure clearly caused an improved anti-microbial effect for the coated
16 catheters.

1

2

3

Table 5

Conditions of DC Magnetron Sputtering Used for Anti-Microbial Coatings

4

5

Samples Sputtered in Commercial Argon

Samples Sputtered in 99/1 wt% Ar/O₂

6

Power 0.1 kW

Power 0.5 kW

7

Target 20.3 cm dia Ag

Target 20.3 cm dia Ag

8

Argon Pressure: 0.7 Pa (5 m Torr)

Ar/O₂ Pressure: 5.3 Pa (40 m Torr)

9

Total Mass Flow: 700 sccm

Total Mass Flow: 700sccm

10

Initial Substrate Temperature: 20°C

Initial Substrate Temperature: 20°C

11

Cathode/Anode Distance: 40 mm

Cathode/Anode Distance: 100mm

12

Film Thickness: 2500 Å

Film Thickness: 3000 Å

13

Example 8

15

This example demonstrates silver coatings formed by arc evaporation, gas scattering evaporation (pressure plating) and reactive arc evaporation. Evaporation of 99.99% silver was performed onto silicon or alumina wafers at an initial substrate temperature of about 21°C, using the parameters as follows:

19 Bias: -100 V

20 Current: 20 Amp-hrs

21 Angle of incidence: 90°

22 Working Gas Pressure: 0.001 Pa (0.01 mT) (arc), 3.5 Pa (26 mT) Ar/H₂ 96:4 (gas scattering evaporation), and 3.5 Pa (26 mT) O₂ (reactive arc evaporation)

24 No corrected ZOI was observed for wafers coated at vacuum (arc). Pressure plating with a working gas atmosphere containing Ar and 4 % hydrogen produced a 6 mm ZOI, while a working gas atmosphere of pure oxygen (reactive arc) produced an 8 mm ZOI. Film thicknesses of about 4000 Angstroms were produced. The results indicate that

- 1 the presence of gases such as hydrogen and/or oxygen in the arc evaporation atmosphere
- 2 cause the coatings to have improved anti-microbial efficacy.

3 **Example 9**

4 This example is included to illustrate composite materials to produce anti-
5 microbial effects. A set of coatings were produced by RF magnetron sputtering zinc oxide
6 onto silicon wafers as outlined below. The zinc oxide coatings showed no zone of
7 inhibition.

8 Coatings of Ag and ZnO were deposited to a total thickness of 3300
9 Angstroms by sequentially sputtering layers of Ag with layers of ZnO, according to the
10 conditions below, in a 75/25 wt% ratio. The coatings were demonstrated to have no zone
11 of inhibition when the zinc oxide layers were about 100 Angstroms thick. However, films
12 consisting of islands of very thin to discontinuous layers of ZnO (less than 50 Angstroms)
13 in an Ag matrix (ie. a composite film) had a 8 mm corrected zone of inhibition.

14 The conditions used to deposit ZnO were as follows:

15 Target 20.3 cm dia Zno; Working gas = argon; Working gas pressure = 4 Pa (30 mT);
16 Cathode-Anode distance: 40 mm; Initial Substrate Temperature: 21°C; Power: RF
17 magnetron, 0.5 kW.

18 The conditions used to deposit the Ag were as follows:

19 Target 20.3 cm dia Ag; Working gas = argon; Working gas pressure = 4 Pa (30 mT);
20 Cathode-Anode distance = 40 mm; Initial Substrate Temperature = 21°C; Power = DC
21 magnetron, 0.1 kW.

1 Example 10

2 This example demonstrates the effects of cold working and annealing silver
3 and gold powders on the anti-microbial efficacy demonstrated by a standard zone of
4 inhibition test. Cold working of such powders results in a defective surface structure
5 containing atomic disorder which favours the release of ions causing anti-microbial
6 activity. The anti-microbial effect of this defective structure can be removed by annealing.

7 Nanocrystalline silver powder (crystal size about 30 nm) was sprinkled onto
8 adhesive tape and tested. A zone of inhibition of 5 mm was obtained, using the method
9 set forth in Example 7. A 0.3g pellet of the nanocrystalline Ag powder was pressed at
10 275,700 kPa (kiloPascal) (40,000 psi). The pellet produced a 9 mm zone of inhibition
11 when tested for anti-microbial activity. Nanocyrstalline silver powder was mechanically
12 worked in a ball mill for 30 sec. The resulting powder was tested for anti-microbial
13 activity, both by sprinkling the worked powder on adhesive tape and applying to the plates,
14 and by pressing the powder into a pellet at the above conditions and placing the pellet on
15 the plates. The zones of inhibition observed were 7 and 11 mm respectively. A pellet that
16 had been pressed from the worked powder was annealed at 500°C for 1 hour under vacuum
17 conditions. A reduced zone of inhibition of 3 mm was observed for the annealed pellet.

18 These results demonstrate that nanocrystalline silver powder, while having
19 a small anti-microbial effect on its own, has an improved anti-microbial effect by
20 introducing atomic disorder by mechanical working of the powder in a ball mill or by
21 pressing it into a pellet. The anti-microbial effect was significantly decreased by annealing
22 at 500°C. Thus, conditions of mechanical working should not include or be followed by
23 conditions such as high temperature, which allow diffusion. Cold mechanical working

1 conditions are preferred to limit diffusion, for example by working at room temperature
2 or by grinding or milling in liquid nitrogen.

3 Silver powder, 1 micron particle size, was tested in a manner similar to
4 above. The Ag powder sprinkled onto adhesive tape and tested for a zone of inhibition.
5 No zone of inhibition was observed. The powder was worked in a ball mill for 30 seconds
6 and sprinkled onto adhesive tape. A 6 mm zone of inhibition was observed around the
7 powder on the tape. When the Ag powder (as is or after mechanical working in the ball
8 mill) was pressed into a 0.3 g pellet using 275,700 kPa (40,000 psi), zones of inhibition
9 of 5 and 6 mm respectively were observed. A pellet which was formed from the ball
10 milled powder and which was annealed at 500°C for 1 hour had significantly reduced anti-
11 microbial activity. Initially the pellet had some activity (4.5 mm zone of inhibition) but
12 after the pellet was tested a second time, no zone of inhibition was observed. A control
13 pellet which had not been annealed continued to give a zone of inhibition greater than 4
14 mm even after 14 repeats of the test. This demonstrates that an annealing step, following
15 by mechanical working, limits the sustainable release of the anti-microbial silver species
16 from the powders.

17 Nanocrystalline gold (20 nm crystals), supplied as a powder, was tested for
18 anti-microbial effect by sprinkling the powder onto adhesive tape and using the zone of
19 inhibition test. No zone of inhibition was recorded for the nanocrystalline gold powder.
20 The gold powder was pressed into a 0.2 g pellet using 275,700 kPa (40,000 psi). A 10
21 mm zone of inhibition was observed. When the pressed pellets were subsequently vacuum
22 annealed at 500°C for 1 hour and the zone of inhibition was found to be 0 mm.

23 The results showed that solubility and thus the anti-microbial efficacy of
24 gold powders can be improved by a mechanical working process such as pressing a

1 nanocrystalline material into a pellet. The anti-microbial activity can be removed by
2 annealing. Cold working is preferred.

3 Other gold powders including a 2-5 micron and a 250 micron particle size
4 powder did not demonstrate an anti-microbial effect under the above mechanical working
5 conditions. It is believed that the small grain size of the nanocrystalline gold powder was
6 an important cofactor which, with the mechanical working, produced the desired anti-
7 microbial effect.

8 Example 11

9 This example is included to demonstrate a composite anti-microbial coating
10 formed by reactive sputtering (another example of composite films). Example 7
11 demonstrates that an anti-microbial coating of silver can be obtained by sputtering in argon
12 and 1% oxygen (0.5 kW, 5.3 Pa (40 mTorr), 100 mm anode/cathode distance, and 20°C -
13 produced a zone of inhibition of 11 mm).

14 When a working gas of argon and 20 wt% oxygen was used to sputter anti-
15 microbial coatings under the conditions listed below, the zones of inhibition ranged from
16 6 to 12 mm. This indicates that the provision of a reactive atmosphere during vapour
17 deposition has the result of producing an anti-microbial film over a wide range of
18 deposition process parameters.

19 Table 6 - Sputtering Conditions

20	Target	20.3 cm dia, 99.99% Ag
21	Working Gas:	80/20 wt% Ar/O ₂
22	Working Gas Pressure:	0.3 to 6.7 Pa (2.5 to 50 mTorr)
23	Total Mass Gas Flow:	700 sccm
24	Power:	0.1 to 2.5 kW
25	Substrate Temperature:	-5 to 20°C
26	Anode/Cathode Distance	40 to 100 mm
27	Base Pressure:	less than 5 x 10 ⁻⁴ Pa (4 x 10 ⁻⁶ Torr)

1 Example 12

2 This example demonstrates that the coatings of this invention have an anti-
3 microbial effect against a broad spectrum of bacteria.

4 A total of 171 different bacterial samples encompassing 18 genera and 55
5 species were provided by the Provincial Laboratory of Public Health for Northern Alberta.
6 These samples had been quick frozen in 20% skim milk and stored at -70°C for periods
7 ranging from several months to several years. Fastidious organisms which were unlikely
8 to grow under conditions used in standard Kirby-Bauer susceptibility testing were not used.

9 Each frozen sample was scraped with a sterile cotton swab to inoculate a
10 blood agar plate (BAP). The plates were incubated overnight at 35°C. The following
11 morning isolated colonies were subcultured onto fresh BAPs and incubated at 35°C
12 overnight. The next day, the organisms were subjected to Kirby-Bauer susceptibility
13 testing as described below.

14 Four to five colonies (more if colonies were small) of the same
15 morphological type were selected from each BAP subculture and inoculated into individual
16 tubes containing approximately 5 mL of tryptic soy broth (TSB). The broths were
17 incubated at 35°C for approximately 2 to 3 hours. At this time, the turbidity of most of
18 the broth cultures either equalled or exceeded that of a 0.5 McFarland standard. The more
19 turbid samples were diluted with sterile saline to obtain a turbidity visually comparable to
20 that of the standard. To aid in the visual assessment of turbidity, tubes were read against
21 a white background with contrasting black line.

22 A small number of the organisms (*Streptococcus* and *Corynebacterium*) did
23 not grow well in TSB. The turbidity of these broths, after incubation, was less than that

1 of the 0.5 McFarland standard. Additional colonies from the BAP subcultures were
2 inoculated to these tubes to increase the turbidity to approximate that of the standard.

3 Within 15 minutes of adjusting the turbidity of the bacterial suspensions a
4 sterile cotton swab was dipped into each broth. Excess fluid was removed by rotating the
5 swab against the rim of the tube. The inoculum was applied to a Mueller Hinton (MH)
6 agar plate by streaking the swab evenly in three directions over the entire agar surface.
7 Three 1 cm x 1 cm silver coated silica wafer squares were applied to each MH plate and
8 the plates were inverted and incubated overnight at 35°C. The coatings had been sputtered
9 under the following conditions, which through XRD analysis were shown to be silver/silver
10 oxide composite films:

11	Target:	20.3 cm dia, 99.99% Ag
12	Working gas:	80/20 wt % Ar/O ₂
13	Working gas pressure:	5.3 Pa (40 mT)
14	Total Mass Gas Flow:	700 sccm
15	Power:	0.1 kW
16	Temperature of Deposition	20°C
17	Base pressure	2.7 X 10 ⁻⁴ Pa (2 x 10 ⁻⁶ Torr)
18	Cathode/anode distance	40 mm

19 BAP cultures of control organisms were provided by the Provincial
20 Laboratory and included: *Staphylococcus aureus* ATCC 25923; *Pseudomonas aeruginosa*
21 ATCC 27853; *Escherichia coli*: ATCC 25922; and *Enterococcus faecalis* ATCC 29212 to
22 check the quality of the MH agar. These cultures were treated in a like manner to the test
23 organisms except that standard antibiotic discs rather than silver coated wafers were
24 applied to the bacterial lawns on the MH agar. These organisms demonstrated that the MH
25 agar was suitable for standard ZOI tests.

26 After 16 to 18 hours of incubation at 35°C zones of inhibition around the
27 silver wafers or antibiotic discs were measured to the nearest mm. Corrected zones were

- calculated by subtracting the size of the wafer (1 cm) from the size of the total zone.
Representative zone of inhibition results are shown in Table 7.

Table 7
The Sensitivity of a Broad Range of Microorganisms to Silver* Coated Silicon Wafers

	Organism	Source	Corrected Zone of Inhibition (mm)
6	<i>Staphylococcus epidermidis</i> RC-455	blood	10
7	<i>Bacillus licheniformis</i> R-2138	tibia	6
8	<i>Corynebacterium sp</i> R-594	leg	10
9	<i>Listeria monocytogenes</i> R-590	blood	5
10	<i>Enterococcus faecalis</i> SR-113	bone	5
11	<i>Streptococcus bovis</i> SR-62	blood	10
12	<i>Escherichia coli</i> R-1878	urine	11
13	<i>Klebsiella ozonae</i> R-308/90	abdomen	10
14	<i>Enterobacter cloacae</i> R-1682	unknown	8
15	<i>Proteus vulgaris</i> 3781	urine	4
16	<i>Providencia stuartii</i> U-3179	urine	8
17	<i>Citrobacter freundii</i> U-3122/90	urine	7
18	<i>Salmonella typhimurium</i> ER-1154	urine	6
19	<i>Serratia marcescens</i> R-850	sputum	6
20	<i>Pseudomonas aeruginosa</i> U-3027	urine	10
21	<i>Xanthomonas malophilia</i> 90-10B	unknown	9
22	<i>Aeromonas caviae</i> R-1211	wound	5
23	<i>Branhamella catarrhalis</i> R-2681	unknown	12
24	Silver deposition*		

46 Example 13

47 This example demonstrates the use of tantalum as an adhesive layer for
48 coatings of this invention. Tantalum is well known as a material which, in the form of an
49 interlayer, improves adhesion of thin films to substrates. In this example test sections

1 including a group of stainless steel (316) (1 x 1 cm) and silicon (1.7 X 0.9 cm) coupons
2 and sections of latex tubing (5 cm) were cleaned in ethanol and then half of the test
3 sections were coated (by sputtering) with a thin layer (approx. 100 Angstroms) of Ta
4 before an anti-microbial silver film was deposited on them. The second group of the test
5 sections were only coated with the anti-microbial Ag film. Coating conditions are listed
6 below. While all test sections had similar anti-microbial activity, the Ta coated test
7 sections had much better adhesion properties than did the untreated test sections. Adhesion
8 properties were determined using ASTM method D3359-87, a standard test method for
9 measuring adhesion.

10 Sputtering Conditions

11	Target:	20.3 cm dia, 99.99% Ta
12	Working Gas:	99/1 wt% Ar/O ₂
13	Working Gas Pressure:	1.3 Pa (10 mTorr)
14	Total Mass Gas Flow:	700 sccm
15	Power:	0.5 kW
16	Cathode/Anode Distance:	100 mm
17	Substrate Temperature:	20°C

18	Target:	20.3 cm dia, 99.99% Ag
19	Working Gas:	99/1 wt% Ar/O ₂
20	Working Gas Pressure:	5.3 Pa (40 mTorr)
21	Total Mass Gas Flow:	700 sccm
22	Power:	0.5 kW
23	Cathode/Anode Distance:	100 mm
24	Substrate Temperature:	20°C

25 Example 14

DC magnetron sputtering was used to deposit silver from a 20.3 cm dia, 99.98% pure cathode onto silicon and alumina wafers with commercial argon moisturized with water as the working gas at a total mass gas flow of 700 sccm. The argon was moisturized by passing it through two flasks containing 3 litres of room temperature water and one empty flask set up with glass wool to absorb any free liquid before the gas entered the sputtering unit.

The conditions of sputtering and the results of the standard zone of inhibition test performed on the sputtered silver films are shown below. Silver films which normally had no anti-microbial properties when deposited using argon that had not been treated with water yielded a corrected zone of inhibition of up to 8 mm when sputtered using a argon/water vapour mixture as the working gas.

Table 8
Conditions used for DC Magnetron Sputtering of Anti-Microbial Coatings

	Working Gas	Working Gas Pressure Pa (mTorr)	Power	Substrate Temperature	Anode/Cathode Distance	Corrected ZOI
14	Commercial Argon	1.3 (10)	0.5kW	-10°C	100 mm	0 mm
15	Ar passed through H ₂ O	1.3 (10)	0.5kW	-10°C	100 mm	8 mm

18 Example 15

19 This example is included to illustrate the method of activating coatings with
20 radiation, in accordance with another aspect of the present invention.

21 A series of 1.9 x 0.7 cm silicon wafers were coated with 3000 Å coatings
22 of silver metal using DC magnetron sputtering under the following conditions:

Sputtering Conditions

24	Target	20.3 cm dia, 99.99% Ag
25	Working Gas	99/1 wt% Ar/O ₂
26	Working Gas pressure	5.3 Pa (40 mTorr)
27	Total Mass Gas Flow	700 sccm
28	Power	0.5 kW
29	Substrate Temperature	21°C
30	Anode/Cathode Distance	100 mm

31 The coated wafers were divided into 4 groups and irradiated with varying doses of gamma
32 radiation - 0, 1, 2 and 4 megarad doses - from a ^{60}Co source at Isomedix Inc., Morton
33 Grove, Ill., U.S.A. The samples were placed generally perpendicular to the incoming

1 radiation. After irradiation, the samples were tested for biological activity (anti-microbial
2 effect) using a standard zone of inhibition test on Mueller Hinton Agar (Difco, Mi) with
3 *S. aureus* (ATCC #25923), as set out in previous examples. The results are summarized
4 in Table 9.

5 **Table 9**
6 Effects of Gamma Radiation on Biological Activiy of Anti-Microbial Coatings

7	Gamma Radiation Dose (megarads)	Corrected Zone of Inhibition (mm)
8	0	11
9	1	14
10	2	17
11	4	20

12 The results generally show a log dose response relationship between the
13 radiation dose and the observed biological response to the wafers. This illustrates that the
14 gamma radiation has further activated the coatings of the present invention to enhance the
15 anti-microbial effect.

16 The experiment was repeated with the anti-microbial films being oriented
17 generally parallel to the incoming radiation. This orientation substantially reduced the
18 level of activation of the anti-microbial coatings, such that no increase in the zone of
19 inhibition was observed relative to controls which had not been irradiated.

20 Example 16

21 This example is included to illustrate activation of the anti-microbial
22 coatings in accordance with the present invention with gamma radiation using a dielectric
23 material adjacent to the material during irradiation.

24 A number of 2.5 cm x 2.5 cm pieces of high density polyethylene mesh
25 (such as used in burn wound dressings) were sputter coated with silver metal under the

1 same conditions as set forth in Example 15 with the exception that the power was 0.1 kW.
2 The coated mesh was then irradiated (perpendicular orientation) as set forth in Example
3 15 at 4 megarads. The biological activity was then tested, as set out in Example 15.
4 Control mesh samples (silver coated, no irradiation) gave a 10mm ZOI(corrected), while
5 the irradiated samples gave a 14 mm ZOI(corrected).

6 Further samples of the coated mesh were irradiated while sandwiched
7 between two 2.5 cm x 2.5 cm silicon wafers having a 1000 Å thermally grown oxide layer,
8 as supplied by the Alberta Microelectronics Centre, Edmonton, Alberta. This mesh sample
9 was tested for biological activity and was found to produce a 26 mm ZOI(corrected).
10 Without being bound by the same, it is believed that the silicon wafers provide a source
11 of electrons which are forward scattered to the anti-microbial coatings, further enhancing
12 the anti-microbial effect.

13 Bulk silver sheet metal was tested to determine whether it could be activated
14 to produce an anti-microbial effect by gamma irradiation. The bulk silver sheet metal
15 samples were annealed at 140°C for 90 minutes in air and then irradiated with a 4 megarad
16 dose. The samples were tested for biological activity, but no ZOI was produced. This
17 result appears to indicate that bulk silver, in its normal ordered crystalline state, has too
18 few atomic defects to be activated in accordance with the process of the present invention.

19 Example 17

20 This example is included to illustrate that anti-microbial coatings containing
21 atomic disorder at a level that is insufficient to produce an anti-microbial effect can be
22 further activated by gamma irradiation, in accordance with the present invention.

1 Silver films were sputtered onto silicon wafers, as set forth in Example 15,
2 except that the gas pressure was reduced from 5.3 Pa (40 mTorr) to 0.7 Pa (5 mTorr),
3 resulting in less atomic disorder in the coatings. The silver films were then irradiated with
4 a 4 Mrad dose of gamma radiation, as in Example 15. The irradiated and control films
5 (not irradiated) were tested for biological activity. The control films produced only 1 mm
6 ZOI(corrected), while the irradiated coatings produced 10 mm ZOI(corrected). This result
7 demonstrates that anti-microbial materials prepared under conditions such that they contain
8 atomic disorder at a level insufficient to produce an anti-microbial effect can be activated
9 so as to be anti-microbial by irradiating with a source of gamma radiation.

10 **Example 18**

11 This example is included to demonstrate the generation of silver complex
12 ions which are distinct from the Ag⁺ ion and which are highly efficacious in generating
13 an anti-microbial effect. The example provides comparative diffusion and zone of
14 inhibition (ZOI) data for various silver solutions.

15 Solutions were prepared to generate 10,000 ppm Ag as AgNO₃, Ag(NH₃)₂⁺,
16 Ag(CN)₂⁻, Ag(S₂O₃)₂³⁻ and Ag(protein).

17 The silver solutions were prepared as follows:

- 18 1) Ag(S₂O₃)₂³⁻ - 2.66 g of AgCl were dissolved in 150 ml of deionized water.
19 17.22 g of Na₂(S₂O₃) were added and the volume was brought up to 200 ml with
20 deionized water.
- 21 2) Ag(CN)₂⁻ - Equal volumes of 12.5 g/L AgCN and 50g/L KCN were mixed.
- 22 3) Ag(protein) - Two silver protein samples were tested. Silver protein powder (0.5
23 g of Sigma S-6767, lot # 121H3437, 20% Ag) were added to 10 ml of deionized

1 water. Silver protein powder (1.25 g of Sigma S-9017, lot # 33H3456, 8% Ag)
2 were added to 10 ml of deionized water.

3 4) $\text{Ag}(\text{NH}_3)_2^+$ - Silver nitrate was added to ammonium hydroxide to form a black
4 precipitate. To this solution was added dropwise additional ammonium hydroxide
5 until the precipitate redissolved, leaving the complex silver ion $\text{Ag}(\text{NH}_3)_2^+$ in
6 solution.

7 Also prepared were control solutions containing the same concentrations of
8 nitrate, ammonia, cyanide and thiosulphate as was present in the test solutions. The anti-
9 microbial effect of the test solutions was tested by a zone of inhibition test. A sensi disc
10 (cellulose, 6mm diameter) containing 25 microlitres of each of the test solutions was
11 placed in the middle of a MHA (Difco media) plate. The silver complexes or ions in the
12 sensi disc were allowed to diffuse for 4 hours on the MHA plate stored in a 37°C
13 incubator. After 4 hours, the sensi disc was removed from the plate and analyzed for
14 silver content using neutron activation analysis (NAA, University of Alberta Slowpoke
15 Reactor Facility). A further set of plates were used to measure zones of inhibition against
16 *S. aureus* for each of the silver complexes or ions in the sensi discs. Samples of the agar
17 were taken from the plates from two locations - the edge of the zone of inhibition and
18 underneath the discs. The agar samples were analyzed for silver content by NAA. The
19 control solutions were tested for anti-microbial effect and were found to cause no zone of
20 inhibition. The results are set forth in Table 10.

Table 10 Anti-Microbial Effect of Ag ⁺ Ion Compared to Silver Complex Ions					
	Test Solution	ZOI (mm)	Silver Content (ppm) <u>In Disc</u>	<u>Under Disc</u>	<u>Edge of ZOI</u>
5	Ag(NO ₃) ₃	6	9000	100	1.8
6	Ag(NH ₃) ₂ ⁺	18	7300	221	1.7
7	Ag(CN) ₂ ⁻	70	1400	420	4.3
8	Ag(S ₂ O ₃) ₂ ³⁻	36	*	*	*
9	Ag(protein)	6	*	*	*
10	* Not measured				

11 The above results indicate that silver salts or compounds known to
 12 dissociate to produce the Ag⁺ ion (ex. silver nitrate and silver proteins) have a limited anti-
 13 microbial effect (6mm ZOI). The anti-microbial effect is greater for silver compositions
 14 which release silver complex ions other than Ag⁺ (ex. Ag(NH₃)₂⁺, Ag(CN)₂⁻ and Ag(S₂O₃)₂³⁻).
 15 It is also apparent that the silver complex ions are able to diffuse further in the agar
 16 medium than the Ag⁺ ion, thereby achieving an anti-microbial effect further from the silver
 17 source.

18 Without being bound by the same, it is believed that the Ag⁺ ion is less
 19 efficacious in its anti-microbial effect because it readily precipitates in the agar medium
 20 with chloride ions known to be present. The silver complex ions on the other hand
 21 demonstrate a higher level of anti-microbial effect and more rapid diffusion. The silver
 22 complex ions are also believed not to precipitate with chloride ions to such an extent,
 23 making them more suitable for use in industrial systems or with medical devices and the
 24 like which come into contact with fluids containing chloride ions.

1 Example 19

2 This example provides comparative diffusion data and zone of inhibition
 3 data for several silver anti-microbial coatings.

4 Three silver films were sputtered under the conditions set forth in Table 11.

5

TABLE 11

	<u>Sputtering Conditions</u>	Film 1	Film 2	Film 3
7	Target (20.3 cm dia)	99.99% Ag	99.99% Ag	99.99% Ag
8	Working Gas	99/1 wt% Ar/O ₂	99/1 wt% Ar/O ₂	99/1 wt%
9	Working Gas Pressure	0.7 Pa	5.3 Pa	5.3 Pa
10	Total Mass Flow	700 sccm	700 sccm	700 sccm
11	Power	0.5 kW	0.5 kW	0.05 kW
12	Substrate Temperature	21°C	21°C	21°C
13	Anode/Cathode Distance	100 mm	100 mm	100 mm

14 The coatings were tested for anti-microbial activity by a ZOI test, as set
 15 forth in previous examples. Silver content was measured by NAA after 4 hours diffusion
 16 in the agar medium, as set forth in Example 18. The comparative results are set out in
 17 Table 12.

18
 19 Table 12
 Anti-Microbial Effect of Silver Coatings

20	Test Film	Ag Species	CZOI (mm)	Silver Content (ppm) Under Film	Silver Content (ppm) Edge of ZOI
21	Film 1	Ag ⁺	2	35	0.8
22	Film 2	AgX ¹	12	8.5	0.7
23	Film 3	Ag ⁺ + AgX ¹	12	654	0.4

26 ¹ AgX is a silver complex ion or ion pair.

27 For Film 1, which releases predominantly Ag⁺ ions, a small ZOI is produced, with the
 28 silver being precipitated as AgCl below the film. For Film 2, a much larger ZOI (6X) is

1 produced with $\frac{1}{4}$ the amount of silver being precipitated under the wafer. This suggests
2 that a silver complex ion different than Ag^+ is formed which diffuses more readily. It is
3 believed that the diffusion is accelerated as a result of the nature of the complex silver
4 species. Film 3 releases much more silver than Films 1 or 2, but the bulk of the silver is
5 in the form of Ag^+ which precipitates as AgCl under the film. However, the size of the
6 ZOI indicates that, in addition to Ag^+ , a complex silver ion with much greater mobility
7 than Ag^+ is generated. It is believed that one or more of the negative silver hydroxyl ions
8 Ag(OH)_2^- , $\text{Ag}_2(\text{OH})_3^-$, or $\text{Ag}_3(\text{OH})_4^-$ are generated. In that chloride is in the agar medium,
9 negative silver hydroxyl-chloro complexes may form.

10 Example 20

11 This example is included to demonstrate the preparation of complex ions of
12 silver cyanide, and the anti-microbial effect of such ions.

13 A silver cyanide bath typically used in electroplating was tested for anti-
14 microbial effect using 25 microlitres of bath on a sensi disc in a standard ZOI test. The
15 silver cyanide bath contained 37 g/L silver cyanide, 45 g/L potassium cyanide and 30 g/L
16 potassium carbonate. The resulting ZOI covered the entire plate, indicating a corrected
17 ZOI greater than 94 mm. The maximum amount of silver that was available in the AgCN
18 bath was 30,000 ppm. From previous work it is known that this concentration as AgNO_3
19 would not yield a ZOI greater than 6 mm. The effect of the cyanide ion alone was
20 determined by placing 25 microlitres of 45 g/L KCN on a sensi disc and repeating the ZOI
21 test. A corrected ZOI of 12.5 mm was produced. A solution of AgCN in distilled water
22 (37 g/L) was similarly tested for a ZOI. A corrected ZOI of 14 mm was observed.

1 The molar ratio of silver ion to cyanide ion in the bath 0.37:1. This favours
2 the formation of a negative silver cyanide complex $\text{Ag}(\text{CN})_2^-$ or AgCN(aq) as an ion pair.
3 The above results demonstrate that these complex silver ions have anti-microbial efficacy
4 and increased mobility within an agar medium.

5 Thin strips of filter paper were treated with 50 microlitres of either a silver
6 nitrate solution (10,000 ppm Ag) or a potassium cyanide solution (6,400 ppm CN). The
7 strips were subjected to a standard ZOI test on the MHA plate. Silver nitrate control strips
8 gave a corrected ZOI of 8 mm, while the KCN control strips gave no ZOI. When one of
9 each of the silver nitrate and potassium cyanide strips were placed on the MHA plate at
10 right angles to each other, the corrected ZOI was 30 mm from the silver nitrate strip and
11 22 mm from the potassium cyanide strip.

12 This result demonstrates that a complex silver ion resulting from the
13 combination of silver nitrate and potassium cyanide in the media has greater anti-microbial
14 efficacy than either solution alone.

15 Example 21

16 This example is included to demonstrate the anti-microbial efficacy of a
17 complex silver ion of silver chloride.

18 Silver chloride was pressed into a 0.2 g pellet at 413,550 kPa (60,000 psi)
19 and tested using a standard ZOI test on MHA plates. An 8 mm zone resulted. A mixture
20 of 0.15 g AgCl and 0.05 g NaCl pressed into a pellet at 60,000 psi and similarly tested.
21 A 24 mm zone was observed.

1 The increased concentration of the available chloride ion favours the
2 formation of the complex silver ion AgCl_2^- , which is demonstrated above to have improved
3 anti-microbial efficacy over AgCl .

4 A silver nitrate solution (10,000 ppm Ag) was tested with sensi discs (25
5 microlitres) in a ZOI test. A 6 mm zone was observed. The same concentration of
6 AgNO_3 was tested on an agar plate which had been supplemented with 5% NaCl. A 20
7 mm zone was observed, indicating improved anti-microbial efficacy. A control plate of
8 agar supplemented with 5% NaCl did not inhibit bacterial growth (*S. aureus*).

9 It is believed that the higher concentrations of the chloride ion favoured the
10 formation of the complex silver ion $\text{Ag}(\text{Cl})_2^-$. This species shows three times the anti-
11 microbial efficacy of Ag^+ from silver nitrate.

12 **Example 22 - Animal Testing - Irritation**

13 A primary skin irritation study was performed on New Zealand White
14 (NZW) rabbits using gauze coated with an anti-microbial metal of this invention. The
15 coating was deposited on a USP type VII gauze using the process conditions of example
16 7 where the working gas was 99/1 wt% Ar/O₂.

17 The coated gauze was placed on abraded and unabraded skin on the side of
18 a New Zealand White rabbit. At 24 h the gauze was removed and the site was graded for
19 erythema and edema at 1, 24 and 48 hours after removal.

20 All animals survived to the end of the study. No erythema, edema or
21 infection was observed on any animal. It was concluded that the gauze did not produce
22 local irritation when placed on the skin of male or female NZW rabbits.

1 Example 23 - Animal Testing - Sensitivity

2 The sensitivity of Hartley Guinea Pigs to USP type VII gauze coated with
3 an anti-microbial metal coating of the present invention was investigated. The gauze was
4 coated as per Example 7 using 99/1 wt% Ar/O₂. The split adjuvant technique was used
5 since the test material was not injectable and the application of dry ice to the induction
6 area most closely simulates the clinical situation.

7 There was no evidence that the coated gauze induced erythema or edema
8 and no infection was observed in any of the animals. All animals survived the study.

9 Application of the coated gauze to the skin of male Hartley Guinea Pigs did
10 not result in local sensitivity when tested by the split adjuvant technique.

11 Example 24

12 This example is included to demonstrate that silver powder/NaCl mixtures
13 produce an anti-microbial effect from complex silver ions believed to be AgCl₂⁻.

14 Pellets of silver powder (1 micron) and NaCl (25%) were pressed at the
15 conditions set out below. The anti-microbial effect was measured by a zone of inhibition
16 test with the pellets. A comparative control of pressed silver powder was also tested for
17 a zone of inhibition. The results are shown in Table 13:

18 Table 13
19 Anti-Microbial Effect of Silver Powder/NaCl

Pellet	Compression kg (lb.)	ZOI
Ag + 25% NaCl	454 (1000)	26 mm
Ag + 25% NaCl	1361 (3000)	20 mm
Ag + 25% NaCl	2268 (5000)	19 mm
Ag powder	454 (1000)	<1 mm

1 Example 25

2 This example illustrates the structural and chemical characteristics of sputter
3 deposited silver films that exhibit good anti-microbial activity (corrected zone of inhibition,
4 CZOI) using the zone of inhibition test as set forth in previous examples. The films were
5 produced by sputtering of a solid 20.3 cm dia planar silver magnetron target onto silicon
6 wafer substrates (100 mm from the target) under the conditions summarized in Table 14.
7 The total mass gas flow was 700 sccm. The ratio of substrate temperature to melting point
8 of silver (1234K), T/T_m , was less than 0.3, the thickness of the film was nominally 3000Å
9 and the angle of incidence in each case was 90° (normal incidence). The characteristics
10 of as deposited silver as well as those that were subsequently annealed (in air at 140°C for
11 90 minutes) are described in this example. The films were characterized in terms of
12 structural (grain size, type of defects, recrystallization) and chemical properties (dopant
13 concentration (wherein dopant refers to atomic %O or oxide content), and electrochemical
14 rest potential). The results are summarized in Tables 15 and 16.

15 The dopant concentration in the film was measured using x-ray
16 photoelectron spectroscopy (XPS) and secondary ion mass spectrometry (SIMS). In the
17 XPS technique a monochromatized Al K α x-ray beam was used as the incident beam. A
18 4kV Ar ion beam was rastered over a 2 mm x 2 mm area in order to remove surface
19 contaminants and expose a fresh surface for XPS analysis. A positive cesium ion beam
20 at 12.5 kV was employed for the SIMS analysis. The dopant concentration computed from
21 XPS and SIMS data is summarized in Tables 15 and 16 for both as deposited and annealed
22 films. It can be seen that one preferred characteristic of biologically active silver films in
23 accordance with the invention is the presence of a dopant. The XPS and SIMS data
24 further showed that the dopant, which in the present case was oxygen or both silver oxide

1 and oxygen, was not chemically bound to the silver atoms in the bulk film. Moreover, the
2 dopant as oxygen was incorporated in such amounts as to exceed the room temperature
3 solid solubility in silver.

4 The grain size of as deposited and annealed films was measured from
5 images taken with a transmission electron microscope (TEM). These data, reported in
6 Tables 10 and 11, demonstrate that anti-microbial active silver films of this invention have
7 an average grain size smaller than 200 nm. Active films, as deposited, had an average
8 grain size less than about 140 nm. The most active films, as deposited, had an average
9 grain size less than 90 nm. In addition, high resolution transmission electron microscopy
10 showed that the onset of recrystallization (T_{rec}) commenced at about 90°C. Grain growth
11 of these fine grained, biologically active films, occurred at temperatures well below 0.33
12 T_m , where T_m is the melting point of silver in degrees K, in particular below 140°C. In
13 general, recrystallization diminished anti-microbial activity. However, coatings with higher
14 levels of silver oxide (coatings 3 and 6) retained anti-microbial activity after annealing.
15 It is believed that the oxide pins sufficient atomic defects so as to retain anti-microbial
16 activity after annealing.

17 The TEM analysis further indicated that biologically active silver films
18 contained a number of growth twins. Upon annealing in air at 140°C for 90 minutes these
19 growth twins disappeared and annealing twins appeared. These latter twins were, however,
20 the result of recovery, recrystallization and grain growth which transformed the silver film
21 into a lower energy state. Evidently, these deposited silver films, along with the associated
22 growth twins that underwent such grain growth, were in a higher energy state. Thus, the
23 presence of these aforementioned defects in the as deposited films is a distinguishing
24 characteristic of anti-microbial coatings in accordance with this invention. Figures 1 and

1 2 are TEM micrographs showing the grain sizes and twins observed in as deposited and
 2 annealed silver films respectively.

3 The rest potential of the silver films was measured in one molar (1M)
 4 potassium hydroxide (KOH) solution using a saturated calomel electrode (SCE) as the
 5 reference electrode. Tables 15 and 16 show that the silver films exhibited anti-microbial
 6 behaviour only when the rest potential was positive. No biological activity was observed
 7 when the rest potential was negative.

8 **Table 14**
 9 Growth Conditions for Sputter Deposited Silver Anti-microbial Coatings

10	11	GROWTH CONDITIONS			
		12	Gas Composition	Pressure Pa (mTorr)	Power(kW)
13	1	99% Ar, 1% O	1.3 (10)	0.10	
14	2	99% Ar, 1% O	1.3 (10)	0.50	
15	3	99% Ar, 1% O	5.3 (40)	0.05	
16	4	99% Ar, 1% O	5.3 (40)	0.10	
17	5	99% Ar, 1% O	5.3 (40)	0.50	
18	6	80% Ar, 20% O	5.3 (40)	0.10	
19					

20 **Table 15**
 21 Structural Characteristics of Sputter Deposited Silver Anti-microbial Coatings

22	23	24	As Deposited						
			Growth Condition	ID Number	Grain Size	Dopant	Rest Potential	Defects	C Z O I
26	27	28	(nm)		Concentration Atomic %O	mV (vs SCE) [†]			(nm)
29	30	31	37	1	5.5	+125		Growth twins	9
32	33	34	148	2	0	-342		-	2
35	36	37	21	3	20.0*	+150		Growth twins	10
38	39	40	19	4	8.0	+135		Growth twins	7
41	42	43	41	5	3.4	+131		Growth twins	9
44	45	46	22	6	58.0*	+146		-	8
Bulk Silver			>200		0	-170		-	<1

* as Ag₂O

[†] These values are subject to variability of ±20 mV
 - not measured

Table 16

Growth Condition		Annealed at 140°C, 90 Minutes			
ID Number	Grain Size	Dopant	Rest Potential	Defects	CZOI
	(nm)	Concentration atomic %O	mV (vs SCE) ¹		(mm)
1	91	-	-6	Annealing twins	1
2	135	0	-224	Annealing twins	0
3	130	16.0*	+121	Annealing twins	10
4	73	0.8	+33	Annealing twins	8
5	132	0.7	-29	Annealing twins	0
6	-	31.0*	+127	-	8
Bulk Silver	>200	0	-170	-	<1

¹ These values are subject to variability of ± 20 mV
not measured.

20 All publications mentioned in this specification are indicative of the level
21 of skill of those skilled in the art to which this invention pertains. All publications are
22 herein incorporated by reference to the same extent as if each individual publication was
23 specifically and individually indicated to be incorporated by reference.

24 The terms and expressions in this specification are used as terms of
25 description and not of limitation. There is no intention, in using such terms and
26 expressions, of excluding equivalents of the features illustrated and described, it being
27 recognized that the scope of the invention is defined and limited only by the claims which
28 follow.

1 Claims:

2 1. A method of forming an anti-microbial material containing one or
3 more anti-microbial metals, said method comprising:

4 creating atomic disorder in a material containing one or more anti-microbial
5 metals under conditions which limit diffusion such that atomic disorder is retained in the
6 material to provide sustained release of atoms, ions, molecules or clusters of at least one
7 of the metals into an alcohol or water based electrolyte at an enhanced rate relative to the
8 material in its normal ordered crystalline state; and

9 irradiating the material with a low linear energy transfer form of radiation
10 such that at least one anti-microbial metal is released at a concentration sufficient to
11 provide a localized anti-microbial effect.

12 2. The method as set forth in claim 1, wherein the anti-microbial metal
13 is selected from the group consisting of Ag, Au, Pt, Pd, Ir, Sn, Cu, Sb, Bi and Zn or alloys
14 or compounds of one or more of these metals.

15 3. The method as set forth in claim 2, wherein the material is a powder
16 or foil of one or more of the metals, and wherein the atomic disorder is formed by cold
17 working of the powder or foil.

18 4. The method as set forth in claim 3, wherein the material is a
19 nanocrystalline powder.

20 5. The method as set forth in claim 2, wherein the material is formed
21 as a coating on a substrate by vapour deposition under conditions which limit diffusion
22 during deposition and which limit annealing or recrystallization following deposition.

23 6. The method as set forth in claim 5, wherein the material is formed
24 by vacuum evaporation, sputtering, magnetron sputtering or ion plating.

1 7. The method as set forth in claim 6, wherein the anti-microbial
2 material is a composite coating formed by co-, sequentially or reactively depositing an
3 anti-microbial metal in a matrix with atoms or molecules of a different material such that
4 atomic disorder is created in the matrix, said different material being selected from one or
5 more of oxygen, nitrogen, hydrogen, boron, sulphur or halogen absorbed or trapped in the
6 matrix from the atmosphere of the vapour deposition, or an oxide, nitride, carbide, boride,
7 halide, sulphide or hydride of either or both of an anti-microbial metal or of an inert
8 biocompatible metal selected from Ta, Ti, Nb, V, Hf, Zn, Mo, Si, and Al.

9 8. The method as set forth in claim 7, wherein the anti-microbial metal
10 is silver and said different material is one or both of silver oxide and atoms or molecules
11 containing oxygen trapped or absorbed in the matrix from the atmosphere of the vapour
12 deposition.

13 9. The method as set forth in claim 5, wherein the coating is formed
14 by magnetron sputtering at conditions such that the ratio of the temperature of the surface
15 being coated to the melting point of the anti-microbial material being deposited is less than
16 about 0.5, and the working gas pressure is greater than about 1.3 Pa (10mT).

17 10. The method as set forth in claim 7, wherein the coating is formed
18 by magnetron sputtering at conditions such that the ratio of the temperature of the surface
19 being coated to the melting point of the anti-microbial material being deposited is less than
20 about 0.5, and the working gas pressure is greater than about 1.3 Pa (10mT).

21 11. The method as set forth in claim 8, wherein the coating is formed
22 by magnetron sputtering at conditions such that the ratio of the temperature of the surface
23 being coated to the melting point of the anti-microbial material being deposited is less than
24 about 0.5, and the working gas pressure is greater than about 1.3 Pa (10mT).

1 12. The method as set forth in claim 1, 3 or 6, wherein the form of
2 radiation is selected from gamma, beta and x-rays.

3 13. The method as set forth in claim 1, 3 or 6, wherein the source of
4 radiation is gamma radiation, used at a dose of greater than about 1 Mrad.

5 14. The method as set forth in claim 1, 3 or 6, wherein the anti-microbial
6 material being irradiated is oriented substantially perpendicular to the incoming radiation.

7 15. The method as set forth in claim 1, 3 or 6, wherein the material is
8 placed adjacent to a dielectric material during irradiation.

9 16. The method as set forth in claim 1, 3 or 6, wherein the material is
10 sandwiched between silicon oxide surfaces during irradiation.

11 17. A method of producing an anti-microbial effect in an alcohol or
12 water based electrolyte, comprising:

13 (a) preparing a silver material such that it forms complex silver ions
14 other than Ag^+ , Ag^{2+} or Ag^{3+} , in an amount so as to produce an anti-microbial effect in
15 contact with an alcohol or a water-based electrolyte that is greater than that produced by
16 an equivalent amount of silver as Ag^+ ; and

17 (b) bringing the silver material into contact with the alcohol or
18 electrolyte to be treated so as to cause the release of the complex silver ions.

19 18. The method as set forth in claim 17, wherein the silver material
20 forms one or more of the complex silver ions $\text{Ag}(\text{CN})_2^-$, $\text{AgCN}_{(\text{aq})}$ (ion pair), $\text{Ag}(\text{NH}_3)_2^+$,
21 AgCl_2^- , $\text{Ag}(\text{OH})_2^-$, $\text{Ag}_2(\text{OH})_3^-$, $\text{Ag}_3(\text{OH})_4^-$ and $\text{Ag}(\text{S}_2\text{O}_3)_2^{3-}$ in contact with an alcohol or a
22 water based electrolyte.

1 19. The method as set forth in claim 18, wherein the silver material is
2 prepared as a powder, solution or suspension containing one or more of the complex silver
3 ions.

4 20. The method as set forth in claim 17, wherein the silver material is
5 prepared by creating atomic disorder in a silver metal under conditions which limit
6 diffusion such that atomic disorder is retained in the material to provide sustained release
7 of a complex silver ion into the alcohol or water based electrolyte at a concentration
8 sufficient to provide a localized anti-microbial effect.

9 21. The method as set forth in claim 20, wherein the material is a
10 powder or foil of silver metal and wherein the atomic disorder is formed by cold working
11 of the powder or foil.

12 22. The method as set forth in claim 20, wherein the silver material is
13 a fine grain or nanocrystalline powder.

14 23. The method as set forth in claim 20, wherein the silver material is
15 formed as a coating on a substrate by vapour deposition under conditions which limit
16 diffusion during deposition and which limit annealing or recrystallization following
17 deposition.

18 24. The method as set forth in claim 23, wherein the coating is formed
19 by vacuum evaporation, sputtering, magnetron sputtering or ion plating.

20 25. The method as set forth in claim 22, wherein the coating is formed
21 by magnetron sputtering at conditions such that the ratio of the temperature of the surface
22 being coated to the melting point of silver is less than about 0.5, and the working gas
23 pressure is greater than about 1.3 Pa (10 mT).

1 26. The method as set forth in claim 24, wherein the silver material is
2 a composite coating formed by co-, sequentially or reactively depositing the silver metal
3 in a matrix with atoms or molecules of a different material such that atomic disorder is
4 created in the matrix, said different material being selected from one or more of oxygen,
5 nitrogen, hydrogen, boron, sulphur or halogen absorbed or trapped in the matrix from the
6 atmosphere of the vapour deposition, or an oxide, nitride, carbide, boride, halide, sulphide,
7 or hydride of silver or of an inert biocompatible metal selected from Ta, Ti, Nb, V, Hf,
8 Zn, Mo, Si and Al.

9 27. The method as set forth in claim 26, wherein the different material
10 is one or more of silver oxide and atoms or molecules containing oxygen trapped or
11 absorbed from the atmosphere of the vapour deposition.

12 28. The method as set forth in claim 17, 18, 19 or 20, wherein the silver
13 material is prepared as an anti-microbial coating on a medical device.

14 29. The method as set forth in claim 17, 18, 19 or 20, wherein the silver
15 material is prepared as a powder for use in the preparation of a topical anti-microbial
16 composition.

17 30. An anti-microbial form of silver material, comprising:
18 silver metal, or an alloy or compound thereof, in a form characterized by
19 sufficient atomic disorder such that the material, in contact with an alcohol or a water
20 based electrolyte, releases atoms, ions, molecules or clusters containing silver on a
21 sustained basis at a concentration sufficient to provide a localized anti-microbial effect,
22 said material being further characterized in that it has a positive rest potential, when
23 measured against a saturated calomel reference electrode, in 1M potassium hydroxide.

- 1 31. The material as set forth in claim 30, wherein the material is further
2 characterized in that the ratio of its temperature of recrystallization to its melting
3 temperature, in degrees K, (T_{rc}/T_m), is less than about 0.33.
- 4 32. The material as set forth in claim 30, wherein the material is further
5 characterized in that the ratio of its temperature of recrystallization to its melting
6 temperature, in degrees K, (T_{rc}/T_m), is less than about 0.3
- 7 33. The material as set forth in claim 31, wherein the material is further
8 characterized in that it has a temperature of recrystallization less than about 140°C.
- 9 34. The material as set forth in claim 33, wherein the material is further
10 characterized in that it has a grain size less than about 200nm.
- 11 35. The material as set forth in claim 33, wherein the material is further
12 characterized in that it has a grain size less than about 140nm.
- 13 36. The material as set forth in claim 33, wherein the material is further
14 characterized in that it has a grain size less than about 90nm.
- 15 37. The material as set forth in claim 34, in the form of a powder, or
16 flakes.
- 17 38. The material as set forth in claim 35, in the form of a nanocrystalline
18 powder.
- 19 39. The material as set forth in claim 34, in the form of a foil.
- 20 40. The material as set forth in claim 34, in the form of a coating.
- 21 41. The material as set forth in claim 34, in the form of a thin film.
- 22 42. The material as set forth in claim 36, in the form of a thin film
23 coating.

1 43. The material as set forth in claim 37, 38 or 42 in the form of
2 substantially pure silver metal.

3 44. The material as set forth in claim 37, 38 or 42, in the form of a
4 mixture of substantially pure silver metal and silver oxide.

5 45. The material as set forth in claim 37, 38 or 42, in the form of
6 substantially pure silver metal and absorbed, trapped, or reacted atoms or molecules of
7 oxygen.

8 46. The material as set forth in claim 45, which further includes silver
9 oxide.

10 47. A fine grain anti-microbial material, comprising:
11 one or more anti-microbial metals, or alloys or compounds thereof, having
12 a grain size less than 200 nm, characterized by sufficient atomic disorder such that the
13 material, in contact with an alcohol or a water based electrolyte, provides a sustained
14 release of atoms, ions, molecules or clusters containing at least one metal at a
15 concentration sufficient to provide a localized anti-microbial effect.

16 48. The anti-microbial material as set forth in claim 47, in the form of
17 a fine grain powder wherein the anti-microbial metal is formed in a matrix with atoms or
18 molecules of a different material, the different material being selected from inert
19 biocompatible metals, oxygen, nitrogen, hydrogen, boron, sulphur, halogen, and oxides,
20 nitrides, carbides, borides, sulphides and halides of either or both of an anti-microbial
21 metal or an inert biocompatible metal.

22 49. The anti-microbial material as set forth in claim 48, wherein the anti-
23 microbial metal is selected from the group consisting of Ag, Au, Pt, Pd, Ir, Sn, Cu, Sb, Bi,

1 and Zn or an alloy or compound thereof, and wherein the biocompatible metal is selected
2 from the group consisting of Ta, Ti, Nb, B, Hf, Zn, Mo, Si, and Al.

3 50. The anti-microbial material as set forth in claim 49, wherein the anti-
4 microbial metal is selected from Ag, Au or Pd, and wherein the biocompatible metal is
5 selected from Ta, Ti or Nb.

6 51. The anti-microbial material as set forth in claim 48, comprising
7 substantially pure silver metal, silver oxide and trapped or absorbed atoms of oxygen.

8 52. The anti-microbial material as set forth in claim 48, 49, 50, or 51 in
9 the form of a nanocrystalline powder having a grain size less than about 20 nm.

10 53. The anti-microbial material as set forth in claim 48, 49, 50 or 51 in
11 the form of a fine grain powder having a grain size less than about 140 nm.

12 54. A method of producing a fine grain anti-microbial material,
13 comprising:

14 depositing one or more anti-microbial metals in a matrix with atoms or
15 molecules of a different material, in a powder form, by vapour deposition onto a cooled
16 substrate, to provide a material having atomic disorder such that the powder, in contact
17 with an alcohol or a water based electrolyte, provides a sustained release of ions, atoms,
18 molecules or clusters of at least one of the anti-microbial metals into the alcohol or water
19 based electrolyte at a concentration sufficient to provide a localized anti-microbial effect,
20 wherein the different material is selected from the group consisting of inert, biocompatible
21 metals, oxygen, nitrogen, hydrogen, boron, sulphur, halogens, and oxides, nitrides,
22 carbides, borides, sulphides and halides of an anti-microbial metal or an inert,
23 biocompatible metal.

1 55. The method as set forth in claim 54, wherein the anti-microbial metal
2 is selected from the group consisting of Ag, Au, Pt, Pd, Ir, Sn, Cu, Sb, Bi and Zn or alloys
3 or compounds of one or more of these metals, and wherein the biocompatible metal is
4 selected from the group consisting of Ta, Ti, Nb, B, Hf, Zn, Mo, Si and Al or alloys or
5 compounds of one or more of these metals.

6 56. The method as set forth in claim 54, wherein the anti-microbial metal
7 is selected from Ag, Au, and Pd, and wherein the biocompatible metal is selected from Ta,
8 Ti, and Nb.

9 57. The method as set forth in claim 56, wherein oxygen is included in
10 the working gas atmosphere during vapour deposition such that atoms or molecules of
11 oxygen are trapped or absorbed in the matrix.

12 58. The method as set forth in claim 57, wherein the anti-microbial metal
13 which is deposited is substantially pure silver metal or silver oxide and wherein oxygen
14 may be included in the working gas atmosphere such that the deposited material includes
15 substantially pure silver metal, and one or both of silver oxide and atoms or molecules of
16 trapped or absorbed oxygen.

17 59. The method as set forth in claim 54, 55 or 56, wherein the material
18 is deposited as a fine grain powder.

19 60. The method as set forth in claim 54, 55 or 56, wherein the material
20 is deposited as a nanocrystalline powder.

21 61. The method as set forth in claim 54, 55 or 56, wherein the material
22 is deposited as a nanocrystalline film.

23 62. An anti-microbial form of silver material, comprising:

1 silver metal, or an alloy or compound thereof, having a positive rest
2 potential, when measured against a saturated calomel reference electrode, in 1M potassium
3 hydroxide and having a ratio of its temperature of recrystallization to its melting
4 temperature, in degrees K, (T_{rec}/T_m), less than 0.33, and which, in contact with an alcohol
5 or a water based electrolyte, releases atoms, molecules or clusters containing silver or a
6 sustained basis at a concentration sufficient to provide a localized anti-microbial effect.

7 63. The material as set forth in claim 62, wherein the material is further
8 characterized in that the ratio of its temperature of recrystallization to its melting
9 temperature, in degrees K, (T_{rec}/T_m), is less than about 0.3

10 64. The material as set forth in claim 62, wherein the material is further
11 characterized in that it has a temperature of recrystallization less than about 140°C.

12 65. The material as set forth in claim 64, wherein the material is further
13 characterized in that it has a grain size less than about 200nm.

14 66. The material as set forth in claim 64, wherein the material is further
15 characterized in that it has a grain size less than about 140nm.

16 67. The material as set forth in claim 64, wherein the material is further
17 characterized in that it has a grain size less than about 90nm.

18 68. The material as set forth in claim 65, in the form of a powder, or
19 flakes.

20 69. The material as set forth in claim 64, in the form of a nanocrystalline
21 powder.

22 70. The material as set forth in claim 65, in the form of a foil.

23 71. The material as set forth in claim 65, in the form of a coating.

24 72. The material as set forth in claim 65, in the form of a thin film.

1 73. The material as set forth in claim 65, in the form of a thin film
2 coating.

3 74. The material as set forth in claim 68, 69 or 73, in the form of
4 substantially pure silver metal.

5 75. The material as set forth in claim 68, 69 or 73, in the form of a
6 mixture of substantially pure silver metal and silver oxide.

7 76. The material as set forth in claim 68, 69 or 73, in the form of
8 substantially pure silver metal an absorbed, trapped, or reacted atoms or molecules of
9 oxygen.

10 77. The material as set forth in claim 76, which further includes silver
11 oxide.



Fig. 2.



Fig. 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 94/00604

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N59/16 A61L2/16 C23C14/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N A61L C23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	GB,A,2 134 791 (VERNON-CARUS) 22 August 1984 see claims 1-4 see page 1, line 29 - line 41 ---	30-77
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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 94/00604

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